







# ORGANIC CHEMISTRY

FOR

## ADVANCED STUDENTS

### PART III

### SYNTHESIS

BY

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## PREFACE TO THE SECOND EDITION

THE object of recasting the former two volumes of the 'Organic Chemistry for Advanced Students' in the three parts in which they now appear has been to group together allied subjects and to link them as far as possible in a consecutive form. As this entailed re-arrangement of the plates, an opportunity was afforded of bringing the subject-matter up to date, and very considerable additions have been made to the contents of the former volumes. As stated in the original preface, the book is not intended to serve as a reference book, but to furnish a general survey of those fundamental principles which underlie the modern developments of this branch of chemistry.

J. B. COHEN.

*March, 1918.*

## PREFACE TO THE THIRD EDITION

Advantage has been taken of the necessity for a reprint to add a number of references to recent literature, which will enable the student to bring his information up to date.

J. B. COHEN.

*July, 1920.*



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# ORGANIC CHEMISTRY

## PART III

### CHAPTER I

#### THE CARBOHYDRATES

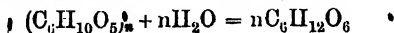
THE carbohydrates are among the principal products of plant life, and they are also elaborated, but to a much smaller extent, in the animal organism. They thus play an important rôle in the economy of nature. Whilst the study of their chemical history within the living organism belongs to the domain of the botanist and physiologist, their chemical behaviour, as denoting structure, claims the attention of the chemist.

The wide and abundant distribution of the vegetable carbohydrates, their extensive consumption as food, their employment in various industries, such as processes connected with fermentation and the manufacture of fabrics and paper, have given them an interest and value which attaches to no other group of compounds.

The carbohydrates include a number of substances, many of them being isomeric, which contain carbon, hydrogen, and oxygen. Though varying widely in physical properties—some, like cane and grape sugar, being sweet, soluble, and crystalline, whilst others, like starch and cellulose, are tasteless, insoluble, and non-crystalline—they are chemically closely related. For the majority of them contain hydrogen and oxygen in the proportion found in water, and hence their composition may be expressed by the general formula  $C_n(H_2O)_n$ , from which the term hydrate of carbon or *carbohydrate* is derived.

It should be added that several recently discovered sugars, for example, rhamnose, fucose, and chinovose (see p. 19), have the formula  $C_6H_{12}O_6$  and form exceptions, but as the term has been generally adopted and still applies to the majority of these compounds, no serious objection can be raised to its use.

The more complex members of the carbohydrates are readily hydrolysed by acids or enzymes into one or more of the simpler members. Thus, starch and cellulose can be converted into glucose.



**Classification of the Carbohydrates.** The large number of the compounds, a number which has been greatly augmented within



last two decades by the masterly researches of Emil Fischer, render it necessary for convenience of study to adopt some method of classification. They fall naturally into two classes, the sweet and crystalline compounds termed *sugars*, and the tasteless and non-crystalline. The non-crystalline carbohydrates possess a more complex structure than the crystalline; but the latter are also divisible into two groups having different molecular formulae.

According to the old system of classification the carbohydrates were divided into three groups, one containing isomeric compounds of the formula  $C_6H_{12}O_6$ , termed *glucoses* or the *grape-sugar* group, a second containing compounds of the formula  $C_{12}H_{22}O_{11}$  and termed *saccharoses* or *cane-sugar* group, and a third containing highly complex compounds of the general formula  $(C_6H_{10}O_5)_n$ , but of unknown molecular weight, termed *amyloses* or *starch* group. The old division is still retained, but the word *glucose* is now reserved for the dextro and laevo enantiomorphs of grape sugar, to replace the older word *dextrose*, which became unsuitable after the discovery of the laevo enantiomorph. For the same reason the word *laevulose* applied to fruit sugar, of which both dextro and laevo varieties are now known, has given place to *fructose*.

The three principal groups of carbohydrates are now distinguished by the names *monosaccharoses*<sup>1</sup> (formerly *glucoses*), *disaccharoses* (formerly *saccharoses*), and *polysaccharoses* (formerly *amyloses*).

After Fischer had succeeded in synthesising a number of new sugars containing more and less than six atoms of carbon, a further subdivision of the monosaccharoses became necessary. The new compounds, containing from two to nine carbon atoms, possess the general characters of monosaccharoses, and must be classified with them. They are distinguished by the names *biose*, *triose*, *tetrose*, &c. Thus, the group of monosaccharoses with six carbon atoms, to which grape and fruit sugar belong, will be termed *hexoses*. Whilst some of the monosaccharoses combine the properties of alcohols and aldehydes, others have the characters of alcohols and ketones, and the additional distinction *aldose* and *ketose* has been introduced. Thus, an aldehyde sugar containing six atoms of carbon would be termed an *aldo-hexose*, whilst the corresponding ketone compound would be a *keto-hexose*.

<sup>1</sup> E. Fischer uses the end syllable 'ide' (e.g. monosaccharido) in place of 'ose' by analogy with the glucosides which are structurally related to the cane-sugar group; but the termination has no significance when applied to members of the grape-sugar group, which are not anhydrides in the same sense that the glucosides are, and therefore the termination adopted above seems on the whole preferable.

**Structure of the Monosaccharoses.** Not more than a quarter of a century ago the monosaccharose group was represented by the four well-known sugars, grape sugar (glucose or dextrose), fruit sugar (fructose or laevulose), galactose, and sorbose. Three of the four substances, namely, glucose, fructose, and galactose, have been the subject of constant and careful study for years past, yet in spite of a vast accumulation of facts their true constitution remained obscure until the years 1885 and 1886, when Kiliani<sup>1</sup> obtained conclusive evidence of their structure.

It had previously been shown that grape sugar, fruit sugar, and galactose yield pentaacetyl derivatives and therefore contain five hydroxyl groups. On reduction they take up two atoms of hydrogen and form hexahydric alcohols, which, with hydriodic acid, are converted into normal, secondary hexyl iodide. Consequently the three sugars consist of a normal chain of six carbon atoms. Five of these are probably present as carbinol groups, since it is unlikely that two hydroxyl groups are attached to one carbon atom. The sixth carbon atom will represent a ketone or aldehyde group; for the sugars undergo reduction and form additive compounds with hydrogen cyanide and combine with hydroxylamine and phenylhydrazine. They all behave more like aldehydes than ketones, inasmuch as they reduce alkaline copper sulphate and silver nitrate solutions; but, on the other hand, whilst glucose yields gluconic acid and galactose yields galactonic acid on oxidation, that is to say, substances which contain the same number of carbon atoms as the original sugars, fructose, under similar conditions, breaks up, and, among the products, trihydroxy-butyric acid has been identified.

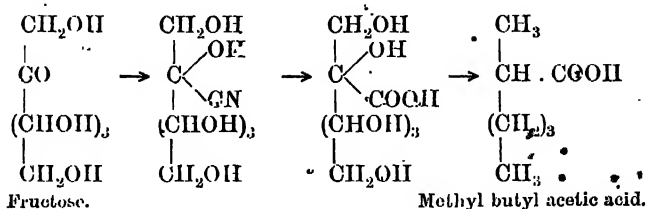
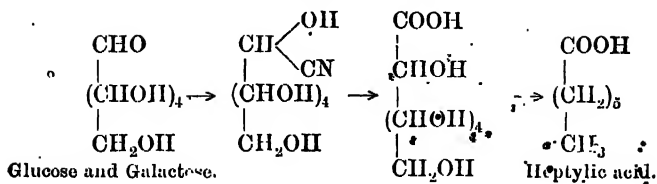
The problem had advanced to this stage when Kiliani published his researches. He hydrolysed the cyanhydrins of glucose, galactose, and fructose, converting them into monobasic acids. They were then reduced with hydriodic acid. Glucose and galactose yielded the same normal heptylic acid, whereas fructose was converted into methyl butyl acetic acid. It was therefore assumed that glucose and galactose are aldehydes and fructose is a ketone, a view which until recently has been accepted. A new theory of their structure which involves the spatial configuration of the molecule has been brought forward by Lowry<sup>2</sup> and E. F. Armstrong<sup>3</sup> and is discussed on p. 42.

<sup>1</sup> *Ber.*, 1885, 18, 3066; 1886, 19, 221, 767, 1128.

<sup>2</sup> *Trans. Chem. Soc.*, 1903, 83, 1314.

<sup>3</sup> *Trans. Chem. Soc.*, 1903, 83, 1305.

## THE CARBOHYDRATES



The abnormal behaviour of fructose in reducing alkaline metallic salts, which is usually regarded as characteristic of aldehydes, is ascribed to the ready oxidisability of hydroxy-ketones. The case is similar to that of the hydroxy-acids, which like tartaric acid are easily oxidised and separate silver from ammonia-silver nitrate, whilst simple dibasic acids like succinic acid have no such action.

In the same year (1887) that Kiliani completed his researches on the constitution of glucose, galactose, and fructose, Emil Fischer<sup>1</sup> prepared the first artificial sugar in the pure state, which he named *α*-acrose. The substance proved to be the inactive representative of natural fructose. Then followed in quick succession the synthesis of ordinary, or dextro-glucose and its optical enantiomorph, laevo-glucose, natural fruit sugar or dextro-fructose, and the corresponding laevo compound and a series of new artificial sugars. The stimulus given by Fischer to this line of research led to a more careful examination of the natural sugars, with the result that dextro-mannose, first prepared artificially by the oxidation of mannitol, was found to be somewhat widely distributed in nature. The monosaccharose group is now represented by sugars which contain from two to ten carbon atoms, members of all the stereoisomeric tetroses, all the aldo-pentoses, and fourteen of the sixteen possible aldo-hexoses, in addition to the isomeric fructoses, sorboses, and other keto-hexoses. The following is a list of the natural and artificial monosaccharoses at present known, all of which have been obtained artificially and their configuration accurately ascertained.<sup>2</sup>

<sup>1</sup> Ber., 1887, 20, 1093, 2566.

<sup>2</sup> Ber., 1891, 27, 3198.

# STRUCTURE OF THE MONOSACCHAROSES .

	<i>Aldoses</i>	<i>Ketoses</i>
<i>Diose</i>	Glycollic aldehyde	
<i>Trioses</i>	<i>d</i> - and <i>l</i> -Glyceric aldehyde	Dihydroxy-acetone
<i>Tetroses</i>	<i>d</i> - and <i>l</i> -Erythrose	<i>d</i> -Erythrulose
	<i>l</i> -Threose	
<i>Methyl tetrose</i>	Rhamnotetrose	
<i>Pentoses</i>	<i>d</i> - and <i>l</i> -Arabinose	<i>l</i> -Arabinulose
	<i>d</i> - and <i>l</i> -Xylose	
	<i>d</i> - and <i>l</i> -Ribose	
	<i>d</i> - and <i>l</i> -Lyxose	
<i>Methyl pentoses</i>	<i>l</i> -Rhamnose	
	<i>d</i> - and <i>l</i> -Isorhamnose	
	<i>d</i> - and <i>l</i> -Fucose (Rhodose)	
	<i>epi</i> -Rhodose <sup>1</sup>	
	Chinovose	
<i>Hexoses</i>	<i>d</i> - and <i>l</i> -Glucose	<i>d</i> - and <i>l</i> -Fructose
	<i>d</i> - and <i>l</i> -Gulose	<i>d</i> - and <i>l</i> -Sorbose
	<i>d</i> - and <i>l</i> -Mannose	
	<i>d</i> - and <i>l</i> -Idose	
	<i>d</i> - and <i>l</i> -Galactose	<i>d</i> -Tagalose
	<i>d</i> - and <i>l</i> -Talose	
	<i>d</i> -Allose	
	<i>d</i> -Altrose	
<i>Methyl hexose</i>	Rhamnoheptose	
<i>Heptoses</i>	<i>d</i> - and <i>l</i> -Mannoheptose	
	$\alpha$ - and $\beta$ -Glucoheptose	
	$\alpha$ - and $\beta$ -Galaheptose	
	Perseulose <sup>2</sup>	
<i>Octoses</i>	Manno-octose	
	$\alpha$ -Gluco-octose	
	Gala-octose	
<i>Nonoses</i>	Mannononose	
	Glucononose	
<i>Decose</i>	Glucodecose <sup>3</sup>	
<i>Aromatic series</i>	Phenyltetrose	

A now and nearly complete chapter has thus been added to organic chemistry, the far-reaching effects of which it is still impossible to forecast. Meanwhile it has afforded a deeper insight into a group of compounds intimately associated with the main function of vegetable life, and at the same time provided a brilliant and convincing proof of the soundness of the van't Hoff-L. Bel hypothesis.

It is not intended to give an abstract of the voluminous literature which has been published on the subject of the monosaccharoses. To do so would be to defeat the object in view, namely, that of placing before the student such facts as will enable him

<sup>1</sup> See footnote, p. 10

<sup>2</sup> Bull. Soc. Chim., 1909, 5, 629

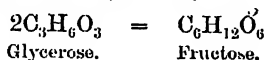
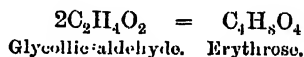
<sup>3</sup> Compt. rend., 1910, 151, 986; 1911, 152, 1774.

to clearly understand the synthetic methods for preparing these substances and the process by which their configuration has been ascertained.

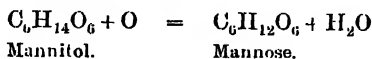
**Natural Sources of the Monosaccharoses.** Some of the monosaccharoses, like glucose and fructose, are found uncombined in plants and animals. These and others may also be obtained from other vegetable products—the glucosides and polysaccharoses—by the hydrolysing action of ferments or acids. Thus, *d* glucose is found in amygdalin, salicin, populin, sinigrin, and in fact in the majority of glucosides, whilst rhamnose is found in quercitrin, fustin, frangulin, &c., and together with galactose in xanthorhamnin. Among the polysaccharoses, cane sugar yields glucose and fructose; raffinose yields glucose, fructose, and galactose, and the cellulose of the ivory nut and other carbohydrates give mannose on hydrolysis.

**Synthetic Preparation of the Monosaccharoses.** The following synthetic methods have been devised for the preparation of the monosaccharoses and, unless otherwise stated, have been elaborated by Emil Fischer.<sup>1</sup>

1. *Polymerisation, or Aldol Condensation, of the lower members of the group by the action of a solution of an alkali.* Glycollic aldehyde has been converted into erythrose and glycerose into fructose (p. 29).



2. *The Oxidation of the Polyhydric Alcohols.* This is effected by means of bromine in presence of sodium carbonate, of nitric acid, or of Fenton's reagent (hydrogen peroxide and a trace of ferrous salt). The product may be an aldehyde or ketone or a mixture of the two. Thus, glycerol gives glycerose (chiefly dihydroxyacetone), whilst mannitol gives the aldehyde, mannose.



3. *The Oxidising action of the 'sorbse bacterium' (bacterium xylinum).* Bertrand<sup>2</sup> found that by the action of the sorbose bacterium, as he named it, the alcohol is converted into the ketose sugar. Glycerol gives dihydroxyacetone, erythritol gives the ketone, erythrulose, arabitol forms arabinulose, sorbitol gives sorbose, mannitol gives fructose, and so forth (p. 38).

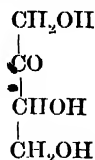
<sup>1</sup> Ber., 1890, 23, 2127; 1894, 27, 3190.

<sup>2</sup> Ann. Chim. Phys., 1904 (8), 3, 181.

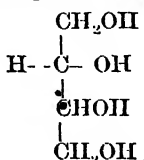
## SYNTHESIS OF THE MONOSACCHAROSES

The action of the organism is selective, depending, as we shall see later (p. 38), on the configuration of the alcohol.

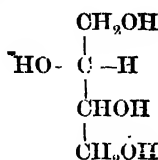
This method also serves the purpose of obtaining active polyhydric alcohols; for on reduction of the ketoses, formed by Bertrand's bacterium, a new asymmetric carbon atom is created which under the asymmetric conditions of its formation produces, or may produce, unequal quantities of active products. Thus, *d*-erythrulose gives on reduction *d*-erythritol as well as the meso product, whilst *d*-sorbose gives *d*-iditol along with *d*-sorbitol.



*d*-Erythrulose.

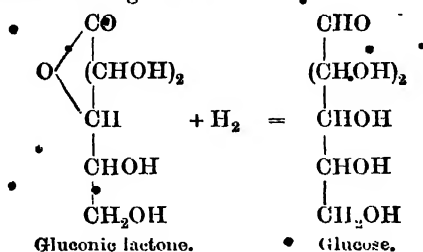


*d*-Erythritol and *i*-Erythritol.

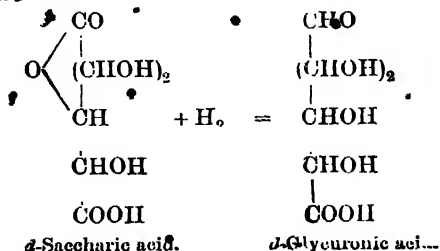


4. *The Reduction of the Lactones of Mono- and Di-basic Hydroxy Acids.* The process was devised by Fischer and is of very general application. It is effected by means of sodium amalgam in a solution maintained slightly acid by the addition of sulphuric acid. The object of keeping the solution acid is to prevent the hydrolysis of the lactone by the formation of the sodium salt which withstands reduction.

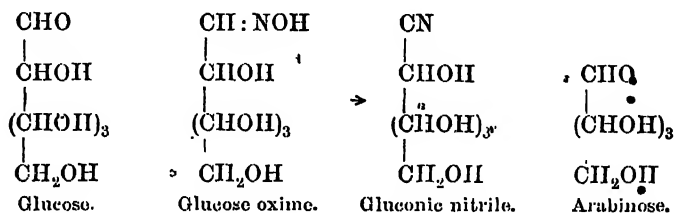
Gluconic lactone forms glucose.



*d*-Saccharic acid can be transformed in the same way into *d*-glycuronic acid.

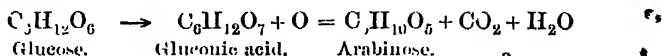


5. *Conversion of a Higher to a Lower Monosaccharose.* This is effected by a method which is due to Wohl<sup>1</sup> and consists in removing water and hydrogen cyanide in the following manner: The monosaccharose, for example, glucose, is converted into the oxime with hydroxylamine. The product is then acted upon with acetic anhydride in presence of sodium acetate, which removes a molecule of water and at the same time acetylates the hydroxyl groups, forming the nitrile of penta-acetylgluconic acid. Ammonia silver nitrate solution now removes hydrogen cyanide and gives the acetyl derivative of the pentose, from which, by the action of ammonia, an acetamide derivative of the pentose is produced. Finally, by the action of sulphuric acid the pentose is liberated.

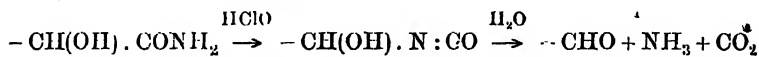


By this method glucose has been converted in successive stages into arabinose, erythrose, glycerose, and glycollic aldehyde.

6. Another method, due to Ruff,<sup>2</sup> produces a similar result. The monobasic acid obtained from the higher monosaccharose is oxidised by means of Fenton's reagent—hydrogen peroxide and a trace of ferrous salt—to the lower sugar. Gluconic acid from *d*-glucose has been converted into *d*-arabinose and *d*-arabinose into *d*-erythrose.



7. Weermann<sup>3</sup> acts upon the lactone of the acid with alcoholic ammonia, which gives the amide, and the latter with hypochlorous acid yields the lower aldose.



8. Löb<sup>4</sup> found that *d*-glucose may be directly oxidised to *d*-arabinose, and formaldehyde by electrolysis in dilute sulphuric acid, using a lead anode.

<sup>1</sup> Ber., 1893, 20, 730; 1897, 30, 3101; 1899, 32, 3666.

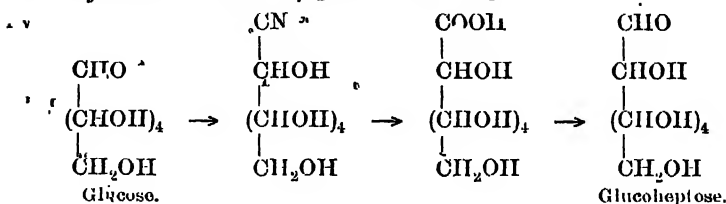
<sup>2</sup> Ber., 1898, 31, 1573.

<sup>3</sup> Biochem. Zeit., 1909, 17, 132, 343.

Abstr., 1915, 2, 137.

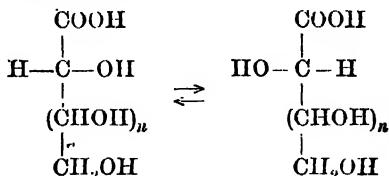
9. Another electrolytic oxidation method has been studied by Neuberg.<sup>1</sup> The sugar is first converted into the copper salt of the monobasic acid, which is then electrolysed, whereby it loses carbon dioxide and hydrogen, and gives the next lower homologue.

10. *Conversion of a Lower to a Higher Monosaccharose.* The method consists in producing the cyanhydrin of the lower sugar, converting the latter into the corresponding acid by hydrolysis, and reducing the lactone as previously described (p. 7). Glucose has been converted into glucoheptose in this way.



It should be pointed out that in the process a new asymmetric carbon acid is introduced which may form two enantiomorphous arrangements and consequently give rise to two products. This is actually the case, for on turning to the table on p. 6 it will be seen that  $\alpha$ - and  $\beta$ -glucoheptoses and  $\alpha$ - and  $\beta$ -galucoheptoses are formed from the corresponding hexoses. On the other hand only one product is obtained from mannose.

11. *Interconversion of Isomeric Aldoses.* The conversion of one monosaccharose into another has been effected in the following way: Fischer found that on heating the monobasic acids (derived from the sugars by oxidation) in aqueous solution with pyridine to a temperature of 130–150° a molecular change occurs. The hydrogen atom and hydroxyl group attached to the carbon atom next to the carboxyl group are interchanged and a new stereoisomeric modification is produced. It occasionally happens that the conversion is complete; but, as the process is reversible, the original and the newly formed product are as a rule present as an equilibrium mixture. The reaction is analogous to the conversion of active into racemic and meso tartaric acid when heated with water (see Part II, p. 187), and may be represented as follows:

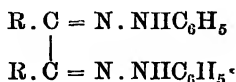


<sup>1</sup> *Biochem. Zeit.*, 1910, 24, 152.

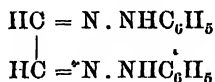


The addition of a weak base-like pyridine prevents the formation of the lactone, which would interfere with the process of conversion. The method is capable of very general application, and has proved of the greatest value, not only in the synthesis of new sugars, but in affording an invaluable means of ascertaining their stereochemical relations. Among the pentoses, arabinose has been converted successively into arabonic acid, then by inversion into ribonic acid, and finally, by reduction of the lactone, into ribose. Xylose has been transformed in the same way into lyxose, whilst in the hexose group mannose has been converted into glucose and galactose into talose. Also a dibasic acid such as mucic acid has been transformed into the stereoisomeric allomucic acid.<sup>1</sup>

12. *Conversion of Aldose into Ketose.* The change of aldose to ketose may be effected by the aid of phenylhydrazine, a reagent which in the skilful hands of E. Fischer has proved invaluable, not only for its present object of converting one sugar into another, but in the more generally useful purpose of isolating, purifying, and identifying the mono- and disaccharoses. All the ketose and aldose sugars combine with one molecule of phenylhydrazine, forming phenylhydrazones. These substances, with the exception of the hydrazone of mannose and a few of the higher members of the group, are very soluble in water. If, however, an excess of phenylhydrazine (at least three molecules), in the form of an aqueous solution of the acetate, is employed, an insoluble yellow crystalline substance is deposited, on warming, which is known as an osazone. Osazones are formed from all compounds containing a ketone and carbinol, aldehyde and carbinol, or two aldehyde or two ketone groups in juxtaposition, and have the general formula :



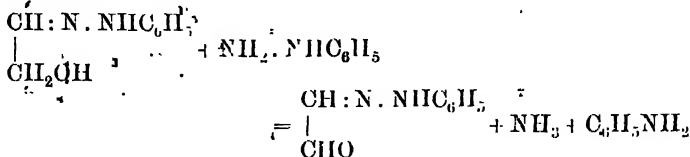
Thus, glycollic aldehyde and glyoxal give the same osazone ;



but in the former case the process takes place in two stages. The phenylhydrazone is first formed and then undergoes oxidation at the

<sup>1</sup> Vatoček has suggested the use of the prefix 'epi' to indicate this mode of derivation. Thus, in place of lyxose the name epi-xylose would be used and lyxose and xylose would be termed 'epimeric'. (*Ber.*, 1911, 44, 819.)

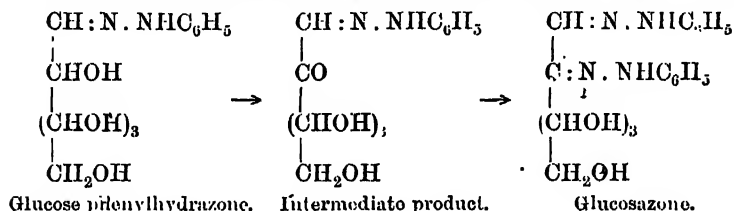
expense of a second molecule of phenylhydrazine, which thereupon breaks up into ammonia and aniline thus:<sup>1</sup>



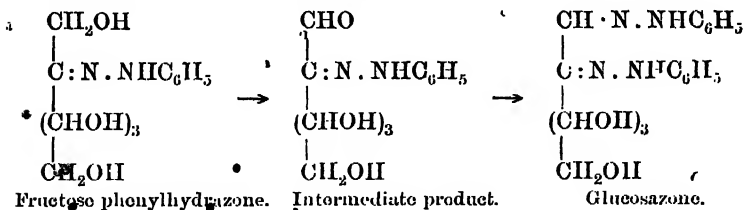
The aldehyde hydrazone then unites with another molecule of phenylhydrazine to form the osazone.



In the case of glucose the changes will be represented as follows:



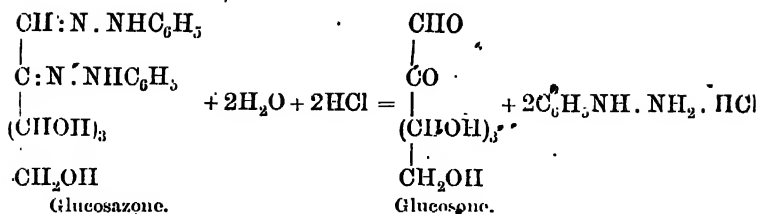
Fructose behaves in a similar fashion.



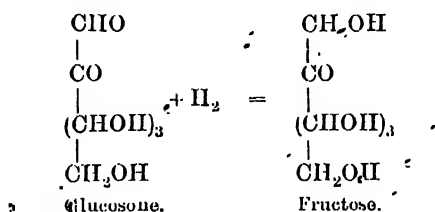
It should be noted that the products obtained from natural glucose and natural fructose are not isomeric but identical, a point of some importance in connection with their space configurations.

Now if the osazone is warmed for a moment with strong hydrochloric acid it is hydrolysed and forms a ketonic aldehyde, which is known as an *osone*, and two molecules of phenylhydrazine are removed.

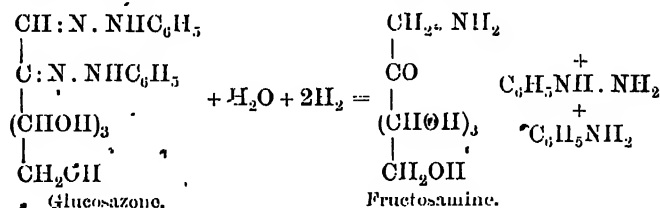
<sup>1</sup> A method of purifying a sugar is to prepare the pure phenylhydrazone (or the bromo- nitro- or diphenyl derivative) and regenerate the sugar by heating with benzaldehyde or formaldehyde, whereby the hydrazone group is transferred to the aldehyde and oxygen takes its place.



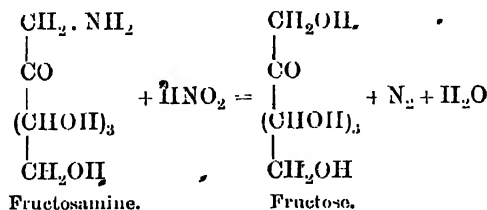
The osone can be isolated in the form of its lead compound, and gives, on reduction with zinc dust and glacial acetic acid, a ketose. In this way glucosone can be converted into fructose.



Another way of compassing the same end is to reduce the osazone with zinc dust and acetic acid when an osamine is formed.



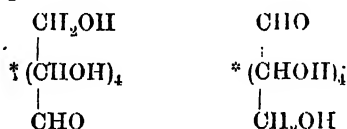
The latter on treatment with nitrous acid then forms the ketose.



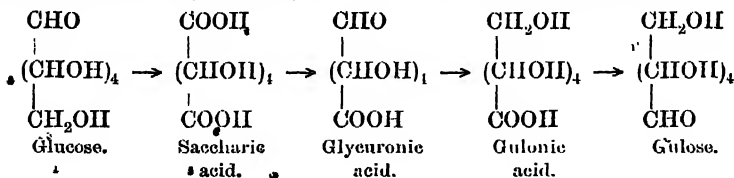
13. *Conversion of Ketose into Aldose.* The only way of producing this change is through the alcohol in the following steps: ketose  $\rightarrow$  alcohol  $\rightarrow$  aldehyde. Thus, fructose on reduction yields a mixture of sorbitol and mannitol, which on oxidation form respectively glucose and mannose. The polyhydric alcohols, it should be added

can be purified in some cases by Meunier's method, which consists in combining the alcohol with benzaldehyde and thus forming a crystalline benzylidene derivative. They also undergo condensation with one, two, and three molecules of acetone, whereby, crystalline mono-, di-, and tri-isopropylidene derivatives are produced, similar in structure to compounds of the sugars (p. 48).<sup>1</sup> Both benzylidene and acetone compounds are readily hydrolysed with dilute acid and yield the polyhydric alcohols.

14. *Inversion of Stereoisomeric Aldoses.* This has been effected by Fischer by the graduated reduction of the dibasic acids. Its significance will be more apparent when the question of configuration is considered. It will be clear, however, that, if the four asymmetric carbon atoms present in glucose have different values, a new stereoisomeric sugar will be formed by interchanging the end carbinol and aldehyde group.



If the lactone of saccharic acid is reduced it forms first the aldehyde acid, viz. glyconic acid, then gulonic acid, in which the original aldehyde becomes a carbinol group; if the lactone of gulonic acid is further reduced, the inverse arrangement of carbinol and aldehyde is finally effected, and the product is known as glucose.



15. *Interconversion of Aldoses and Ketoses.* Lobry de Bruyn and van Ekenstein<sup>2</sup> found that under the influence of alkalis, alkaline earths, sodium acetate, lead oxide, guanidine,<sup>3</sup> &c., the hexoses are slowly transformed into mixtures of their isomers. Each of the hexoses, glucose, fructose, and mannose, forms under these conditions a certain proportion of the other two, together with certain other sugars, e. g. glucose, a  $\gamma$ -ketose, which is also found in molasses.

<sup>1</sup> Fischer, *Ber.*, 1894, 27, 1536; 1895, 28, 1167, 2169; Irvine, *Trans.*, 1914, 105, 898; 1915, 107, 837.

<sup>2</sup> The four asymmetric carbon atoms.

<sup>3</sup> *Ber.*, 1896, 29, 3078; *Rec. trav. chim. Pays-Bas.*, 1900, 19, 1; 1908, 27, 1.

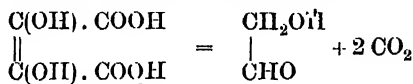
<sup>4</sup> Morell and Bellairs *Trans. Chem. Soc.*, 1907, 91, 1010.

## THE CARBOHYDRATES

*D*-Galactose under similar conditions yields *D*-talose and also two ketoses,  $\psi$ -tagatose which has been identified as *L*-sorbitose, and *D*-tagatose which gives the same osazone as *D*-galactose. *D*-glucose and *D*-idose give *D*-sorbitose, and  $\psi$ -fructose is obtained in the same way from ordinary fructose, and *L*-arabinose is partially transformed into *L*-ribose. The explanation of these changes will be discussed later.<sup>1</sup>

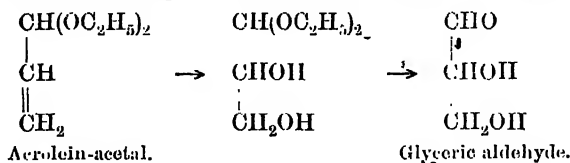
The following is a short account of the principal monosaccharoses.

**Biose.** Glycollic aldehyde is the only biose. Fischer originally prepared it in an impure state by the action of cold baryta water on bromoacetaldehyde. More recently Feiten<sup>2</sup> has obtained it in the crystalline form by heating dihydroxymaleic acid in pyridine to 60°.

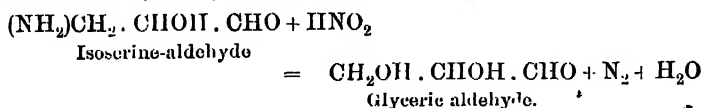


It gives the general reactions of the sugars, and with sodium hydroxide polymerises to a tetrose.

**Trioses.** Fischer and Tafel found that a mixture of glyceric aldehyde and dihydroxyacetone was obtained by oxidising glycerol with dilute nitric acid or with bromine and sodium carbonate. It forms a syrup which polymerises in presence of sodium hydroxide solution, yielding  $\alpha$ -acrose (see p. 19). Pure, inactive glyceric aldehyde was subsequently obtained by Wohl<sup>3</sup> by carefully oxidising acrolein-acetal with cold permanganate solution and then hydrolysing.



The two active compounds were prepared from the corresponding isoserine aldehydes by the action of nitrous acid.<sup>4</sup>



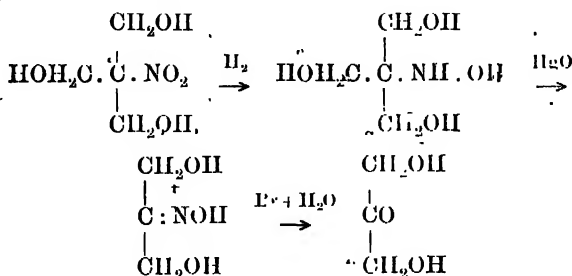
Glyceric aldehyde is characterized by the formation of an insoluble, crystalline substance with phloroglucinol dissolved in hydrochloric

<sup>1</sup> The use of the capitals *D* and *L* have a special significance which is explained in the foot-note p. 17.

<sup>2</sup> *Trans. Chem. Soc.*, 1894, 65, 899; 1895, 67, 48, 774; 1896, 69, 546; 1897, 71, 375; 1899, 87, 804; 1905, 87, 804.

<sup>3</sup> *Ber.*, 1898, 31, 1796, 2394; 1900, 33, 3095; 1914, 47, 3346; see also Witzemann, *J. Amer. Chem. Soc.*, 1915, 36, 1908, 2223. <sup>4</sup> *Ber.*, 1914, 47, 3346.

acid, which the ketone does not give. As the product obtained by Fischer and Tafel gives no reaction with phloroglucinol, it probably contains no aldehyde (see p. 20). Dihydroxyacetone has been obtained by Piloty<sup>2</sup> from formaldehyde and nitromethane in the following way. Condensation of three molecules of aldehyde with one of nitromethane is first effected:



Careful reduction converts the nitro compound into the hydroxylamine derivative, and subsequent oxidation with mercuric oxide yields the oxime of dihydroxyacetone, which is then liberated by oxidation with bromine and water. The ketone is also obtained by the action of Bertrand's *sorbose bacterium* on glycerol (see p. 38).<sup>3</sup>

**Tetroses.** An impure product was originally obtained by the oxidation of erythritol (a tetrahydric alcohol found in certain lichens), and also by the polymerisation of glycollic aldehyde. Both products were inactive. More recently Wohl and Ruff have been successful in obtaining three of the four possible active forms from the active pentoses. Thus the tetrose known as *D*-threose was obtained by Wohl<sup>4</sup> from *D*-xylose, and *L*-erythrose from *L*-arabinose, whilst Ruff<sup>5</sup> prepared *D*- and *L*-erythrose by oxidation of *D*- and *L*-arabonic acid and *D*-threose from *D*-xylonic acid. All the tetroses yield erythritols on reduction and mono- and di-basic acids on oxidation. Whereas the tetroses contain two dissimilar asymmetric carbon atoms, and can therefore form two pairs of enantiomorphs, the alcohols and dibasic acids (tartaric acids), which contain two similar asymmetric carbon atoms, can only produce two active (racemic) and one meso form. Natural erythritol represents the meso form, for it is obtained by the reduction of *D*- and *L*-erythrose, which in turn yield mesotartaric acid on oxidation, whilst Griner's<sup>6</sup> erythritol, which he prepared from butadiene tetrabromide, is the racemic

<sup>1</sup> *Ber.*, 1897, 30, 3161.

<sup>2</sup> *Ber.*, 1899, 32, 3666.

<sup>3</sup> *Compt. rend.*, 1898, 126, 842, 984.

<sup>4</sup> *Ber.*, 1899, 32, 3672; 1901, 34, 1370

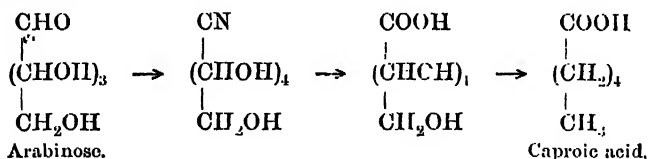
<sup>5</sup> *Compt. rend.*, 116, 723; 117, 553.

modification. The following table represents these relations; where the source is not mentioned it implies that the substance is obtained from the most closely related product by oxidation or reduction.

<i>Tetroses.</i>			
Tetritol.	Aldo-tetrose.	Tetronic acid.	Tartaric acid
<i>i</i> -erythritol (natural)	<i>D</i> -erythrose, (from <i>D</i> -arabinose) <i>L</i> -erythrose (from <i>L</i> -arabinose)	<i>D</i> -erythronic acid	<i>i</i> -tartaric acid
<i>L</i> -erythritol (from erythrulose)			<i>Ld</i> -tartaric acid <sup>1</sup>
<i>D</i> -erythritol	<i>D</i> -threose (from <i>D</i> -xylose)		<i>D</i> -tartaric acid

In addition to the above there is a methyl tetrose which has been prepared by Wohl's method from rhamnose, and a phenyl tetrose which Fischer and Stewart<sup>1</sup> obtained from phenyltrihydroxybutyric lactone by reduction.

*Pentoses.* The pentoses contain three asymmetric carbon atoms, and can therefore exist in eight stereoisomeric forms. All these are known, namely, *D*- and *L*-arabinose, *D*- and *L*-ribose, *D*- and *L*-xylose, and *D*- and *L*-lyxose. *L*-Arabinose is a vegetable product and was first obtained in 1869 by Scheibler, who gave it the formula  $C_5H_{12}O_5$  and included it among the glucoses. Subsequently Kiliani,<sup>2</sup> by a careful analysis of the osazone, showed conclusively that it had the formula  $C_5H_{10}O_5$  and was a pentose. He also prepared the cyanhydrin and by hydrolysis, followed by reduction, transformed it into normal caproic acid, a further proof of the correctness of his analytical results.



Arabinose is prepared from gum-arabic, cherry-gum, and certain other vegetable gums by boiling with dilute sulphuric acid, precipitating the acid with lime, filtering, evaporating to dryness, and extracting the residue with alcohol. On evaporation of the alcohol, arabinose crystallizes in colourless needles which are strongly dextro-rotatory in solution. Arabinose is therefore not present as such in

<sup>1</sup> Ber., 1892, 25, 2555.

Jer., 1887, 20, 353.

the gum, but in a higher complex known as a pentosan, which probably bears the same relation to arabinose that starch does to glucose. It may appear anomalous to denote by the expression '*L*-arabinose' a strongly dextrorotatory compound; but it must be remembered that the terms laevo and dextro, when applied to the sugars, have lost their original meaning, being no longer used to indicate optical character but stereoisomeric relations, in accordance with a suggestion of Fischer. Since, as we shall shortly see, ordinary arabinose is directly related to *L*-glucose, the former, in spite of its dextro-rotatory character, is termed *L*-arabinose.<sup>1</sup> The optical enantiomorph, *D*-arabinose, is obtained from grape sugar by Wohl's and Ruff's methods.

Ribose, the third isomer, is prepared by the reduction of ribonic lactone, the latter being obtained by inversion from *L*-arabonic acid by heating with pyridine in the manner already indicated.

*L*-arabinose  $\rightarrow$  *L*-arabonic acid  $\rightarrow$  *L*-ribonic acid  $\rightarrow$  *L*-ribose.

The enantiomorph *D*-ribose has been obtained by Lovene and Jacobs from the nucleic acid of animal and vegetable cells<sup>2</sup> and by van Ekenstein and Blanksma by the inversion of *D*-arabonic acid.<sup>3</sup>

A second naturally occurring pentose is known as *D*-xylose and was discovered in 1886 by F. Koch. It is isomeric with arabinose and is prepared from wood-gum, a substance which forms part of the woody cell-wall of many plants, in which it is present in the form of a polysaccharose or pentosan. *L*-Xylose is obtained by Wohl's method from *L*-glucose. *D*-Xylose, the seventh isomer, is obtained from *D*-xylose in precisely the same manner as that by which ribose is derived from arabinose. Both *D*- and *L*-xylose have also been prepared from *D*- and *L*-galactose by Wohl's and Ruff's methods.<sup>4</sup> In

<sup>1</sup> According to the system adopted by Fischer, the designations dextro and laevo (*d*, *l*) are used to denote, not the sign of rotation, which may be in either sense, but the relationship of the sugar to dextro- and laevo glucose, to which an arbitrary configuration is given. Thus, ordinary fructose, which is laevo-rotatory, is termed dextro-fructose because it is related in configuration to dextro-glucose, and in the same way natural arabinose, which is dextro-rotatory, is termed laevo-arabinose on account of its relation to laevo-glucose. It is clear, therefore, that two symbols are required, a name or symbol for the family and a second symbol for the rotation. To avoid the confusion which the use of one symbol involves, the large italics *L* and *D* are used to indicate the family and the small italics the rotation. All derivatives from *L* and *D* glucose in the tables will belong to *L* and *D* families respectively, irrespective of their actual rotations, the meso compounds only being distinguished by the symbol *m* and the inactive mixtures by *dl*. Where such a symbol as *DL* occurs it implies that the substance, though laevo-rotatory, belongs to the *D* family. The symbols  $\alpha$  and  $\beta$  are explained later (p. 42).

<sup>2</sup> *Ber.*, 1909, 42, 3247; 1910, 43, 3147.

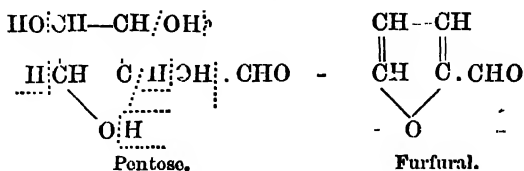
<sup>3</sup> *Chem. Weekblad*, 1913, 1C, 664.

<sup>4</sup> van Ekenstein and Blanksma, *Chem. Weekblad*, 1914, 11, 189.



determining the configuration of the sugars, it is of fundamental importance to remember that ribose and xylose give different but inactive trihydroxyglutaric acids on oxidation, whilst those from *D*- and *L*-arabinose are active.

The pentose sugars are readily distinguished from the other monosaccharoses by boiling with strong hydrochloric acid, which converts them into furfural. The change may be represented thus :



Tollens' reagent (a solution of phloroglucinol or orcinol in strong hydrochloric acid, producing, on warming, a deep cherry-red or violet coloration according to the phenol used) is a useful qualitative test for a pentose.

The following table contains a list of the known pentoses and substances related to them :

*Pentoses.*

Pentitol.	Aldo-Pentose.	Pentonic acid.	Trihydroxy-glutaric acid.
<i>D</i> -xylitol	<i>D</i> -xylose (natural)	<i>D</i> -xylonic acid	<i>D</i> -xylo-trihydroxy-glutaric acid <i>m. p.</i> 152°
<i>D</i> -arabitol	<i>L</i> -xylose (from <i>L</i> -gulose)	<i>D</i> -lyxonic acid	
"	<i>D</i> -lyxose (from <i>D</i> -xylonic acid and <i>D</i> -galactose)	—	—
<i>D</i> -arabitol	<i>L</i> -lyxose (from <i>L</i> -galactose)	<i>D</i> -arabonic acid	<i>D</i> -trihydroxy-glutaric acid <i>m. p.</i> 127°
<i>L</i> -arabitol	<i>D</i> -arabinose (from <i>D</i> -glucose)	<i>L</i> -arabonic acid	<i>L</i> -trihydroxy-glutaric acid <i>m. p.</i> 127°
<i>D</i> -adonitol (from <i>adonis vernalis</i> (Merck))	<i>L</i> -arabinose (natural and from <i>L</i> -glucose)	<i>L</i> -ribonic acid	<i>D</i> -ribo-trihydroxy-glutaric acid <i>m. p.</i> 170–171°
—	<i>D</i> -ribose (from <i>L</i> -arabinose)	—	—
—	<i>D</i> -ribose (from nucleic acid)	—	—

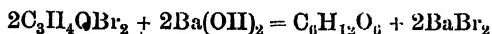
Several methyl pentoses are known and are found in the table on p. 5. They include *L*-rhamnose, a constituent of various glucosides (p. 6), *D*- and *L*-isorhamnose, one of which was obtained by inversion

of rhammonic acid and the other from purgic acid (a constituent of convolvulin); *L-fucose*, which is found as a glucoside in seaweed, and its *D*-enantiomorph, termed also *rhodose*, found in the glucoside, convolvulin along with *D*-isorhamnose. *Epi-rhodose* is obtained from rhodose by inversion; and *chinovose*, which occurs as an ethyl glucoside in chinovite, of cinchona bark. All these substances, on heating with hydrochloric acid, are converted into methyl furfural.

*Hexoses.* The properties of the hexoses are included in the general account of the monosaccharoses already given. They differ, however, from the majority of the other sugars of the group in two important respects. When boiled with dilute mineral acids they form levulinic acid, and, together with glycerose and manno-nonose, are the only sugars which undergo fermentation with yeast. The subject of fermentation is more fully discussed on p. 36.

The history of the synthesis of the hexoses begins with an observation of Batlerow<sup>1</sup> in the year 1861. He found that by the addition of lime-water to a hot solution of trioxymethylene (a solid substance produced by the polymerisation of formaldehyde) *methylenitan* is formed, which is described as a sweet yellow syrup, giving the ordinary reactions for sugar, but optically inactive and incapable of fermentation. A considerable advance was made when Loew<sup>2</sup> discovered that formaldehyde and lime-water at the ordinary temperature yield a sweet syrup of the formula  $C_6H_{12}O_6$ , which he termed *formose*; but this also was unfermentable. A special interest attaches to this reaction, since it gave substantial support to a theory advanced by Baeyer,<sup>3</sup> that the carbon dioxide, assimilated by the plant as starch or sugar, may pass through the stage of formaldehyde. Shortly afterwards Loew<sup>4</sup> modified his method by replacing the lime by magnesia, and obtained a syrup which underwent fermentation. The new product was called *meliose*. All three substances appear from Fischer's subsequent investigations to be complex mixtures containing  $\alpha$ -acrose, which Fischer and Tafel<sup>5</sup> had meantime obtained in a state of purity by entirely different methods.

By the action of baryta on acrolein bromide a mixture was obtained from which two sugars, named  $\alpha$ - and  $\beta$ -acrose, were isolated in the form of their osazones.



This method was afterwards modified by substituting glycerose (obtained from glycerol by oxidation (p. 14)), which, under the action

<sup>1</sup> *Annalen*, 1861, 120, 295; *Compt. rend.*, 1861, 53, 145.

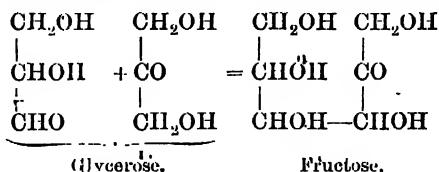
<sup>2</sup> *J. pract. Chem.*, 1886, 33, 321.

<sup>4</sup> *Ber.*, 1889, 22, 475.

<sup>3</sup> *Ber.*, 1870, 3, 67.

<sup>5</sup> *Ber.*, 1887, 20, 1093, 2566.

of dilute alkali, polymerises.<sup>1</sup> The product is a syrup from which  $\alpha$ -acrose can be separated in the form of the crystalline osazone.  $\alpha$ -Acrose<sup>2</sup> was subsequently identified as inactive fructose, whilst  $\beta$ -acrose has been shown to be *dl*-sorbitose,<sup>3</sup> and the reaction was explained by Fischer as taking place in the following way:



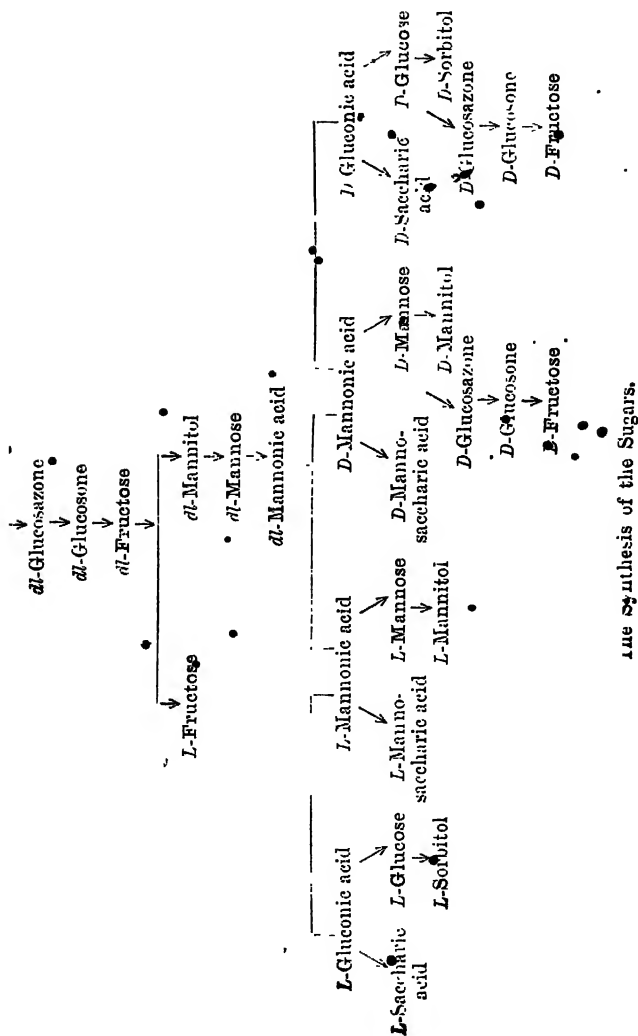
As, according to Wohl,<sup>4</sup> little, if any, glyceric aldehyde is present in Fischer's glycerose, which consists, therefore, almost wholly of dihydroxyacetone, the action of the alkali must produce partial intramolecular change from ketone to aldehyde. The process by which a ketose is transformed into an aldose is quite consistent with observations of Lobry de Bruyn, who noticed that any one of the sugars, fructose, glucose, or mannose, forms under the influence of an alkali an equilibrium mixture of the three (see p. 13). The  $\alpha$ -acrosazone, which was separated from Fischer's product, and closely resembled glucosazone, was converted into the osone and finally into the pure ketose. The latter proved to be the inactive form of fructose. By partial fermentation with yeast the *D*-enantiomorph or ordinary fructose is consumed, and *L*-fructose remains and may be separated. On reduction of inactive fructose, inactive mannitol is formed which, on oxidation, yields inactive mannose and inactive mannonic acid. The latter can be separated into the optical enantiomorphs by fractional crystallization of the strychnine or morphine salts. Thus a *d*- and *l*-mannonic acid are produced, each of which undergoes molecular change on heating with pyridine, being transformed respectively into *d*- and *l*-gluconic acid. The lactones of all the four acids can, on the one hand, be reduced to the corresponding sugars or, on the other, oxidised to the dibasic saccharic acids. In this way two mannoses,<sup>5</sup> two glucoses, and four saccharic acids were prepared artificially by Emil Fischer. Natural fructose, although laevo-rotatory, is *D*-fructose, since it is related to *D*-glucose; for both *D*-glucose and ordinary fruit sugar yield the same *D*-gluco-

<sup>1</sup> Ber., 1887, 20, 3384.

<sup>2</sup>  $\alpha$ -Acrose may also be obtained by the condensation of three molecules of glycollic aldehyde (Fenton), and it is stated that  $\alpha$ -acrose is also formed by the action of ultraviolet light or sunlight on an alkaline solution of glycerol (Compt. rend., 1911, 152, 535). <sup>3</sup> Schmitz, Ber., 1913, 46, 2327. <sup>4</sup> Ber., 1900, 33, 3095.

<sup>5</sup> See also Hudson and Sawyer, J. Amer. Chem. Soc., 1917, 39, 470.

# SYNTHESIS OF THE MONOSACCHAROSES



sazone, and the latter can be converted through the osone into ordinary fructose (p. 11). The same *D*-glucosazone is also given by *L*-mannose, which consequently may be likewise converted into ordinary fructose.

This close relationship between the three natural sugars, *D*-glucose, *D*-fructose, and *D*-mannose, has a peculiar interest from the fact of their occurrence side by side in nature as well as from their stereochemical connection, which will be discussed presently. The tabulated scheme on p. 21 represents the various synthetic steps described above. The sugars are in thick type.

In addition to the two glucoses and two mannoses, eight other stereoisomeric aldoses are known, together with their reduction and oxidation products and numerous other derivatives. They have been obtained as follows:

*D*- and *L*-*Gulose* were prepared by the inversion of *L*- and *D*-glucose by oxidation to the saccharic acids and subsequent reduction as described on p. 13.

*D*- and *L*-*Idose* were obtained from *D*- and *L*-gulonic acid, which, by inversion with pyridine, yield the corresponding *D*- and *L*-idonic acids. *D*-Idonic acid can also be obtained with *D*-gulonic acid from *D*-xylose, which forms the stereoisomeric cyanhydrins (see p. 9).

*D*- and *L*-*Galactose*. If natural or *D*-galactose from milk-sugar is oxidised, it yields meso-mucic acid. If the lactone of the latter is reduced, monobasic galactonic acid is formed, which is racemic and can be resolved into its enantiomorphs by the aid of the strychnine salt.

Each of the active galactonic acids yields an active galactose on reduction.

*D*- and *L*-*Talose*. *D*- and *L*-galactonic acid are converted by inversion with pyridine into *D*- and *L*-talonic acid, which on reduction give *D*- and *L*-talose. Each of these sugars gives a talomucic acid on oxidation. The laevo acid has also been obtained by the oxidation of  $\beta$ -rhamnohexonic acid (p. 34). Allomucic acid was prepared from mucic acid by inversion with pyridine. *D*-*Allose* and *D*-*altrose* have been prepared by Levene and Jacobs<sup>1</sup> from *D*-ribose by the cyanhydrin synthesis. As the former yields allomucic acid on oxidation and *D*-talitol on reduction,<sup>2</sup> the configuration of both can be ascertained.

The following table contains a list of hexoses and related compounds:

<sup>1</sup> *Ber.*, 1910, 43, 3141.

<sup>2</sup> Bertrand and Bruneau, *Compt. rend.*, 1908, 146, 432.

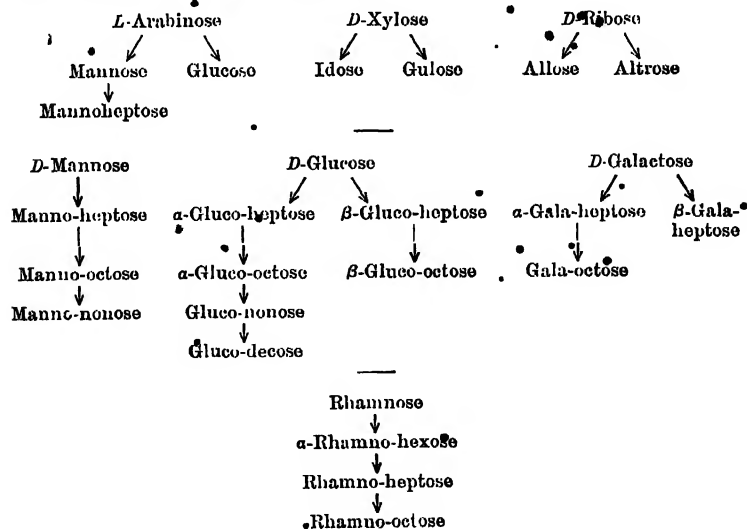
## Aldo-hexoses.

Hexitol.	Aldo-hexose.	Hexonic acid	Tetroxyadipic acid.
<i>D, L</i> -Mannitol	<i>D, L</i> -Mannose	<i>D, L</i> -Mannonic acid	<i>D, L</i> -Manno-saccharic acid
<i>D, L</i> -Iditol	<i>D, L</i> -Idose	<i>D, L</i> -Idonic acid	<i>D, L</i> -Ido-saccharic acid
<i>D, L</i> -Sorbitol	<i>D, L</i> -Glucose	<i>D, L</i> -Gluconic acid	<i>D, L</i> -Saccharic acid
<i>i</i> -Dulcitol	<i>D, L</i> -Gulose	<i>D, L</i> -Gulonic acid	<i>i</i> -Mucic acid
<i>D, L</i> -Talitol	<i>D, L</i> -Galactose	<i>D, L</i> -Galactonic acid	<i>D, L</i> -Talomucic acid
—	<i>D, L</i> -Talose	<i>D, L</i> -Talononic acid	<i>i</i> -Allo-mucic acid
—	<i>D, L</i> -Altrose	<i>D, L</i> -Altronic acid	—
—	<i>D</i> -Allose	<i>D</i> -Allonic acid	—

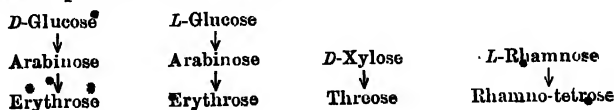
## Keto-hexoses.

<i>D</i> -Mannitol	<i>D</i> -Fructose	—	—
<i>D</i> -Sorbitol	<i>L</i> -Fructose	—	—
<i>D</i> -Sorbitol	<i>D, L</i> -Sorbosc	—	—
—	<i>D</i> -Tagatose	—	—

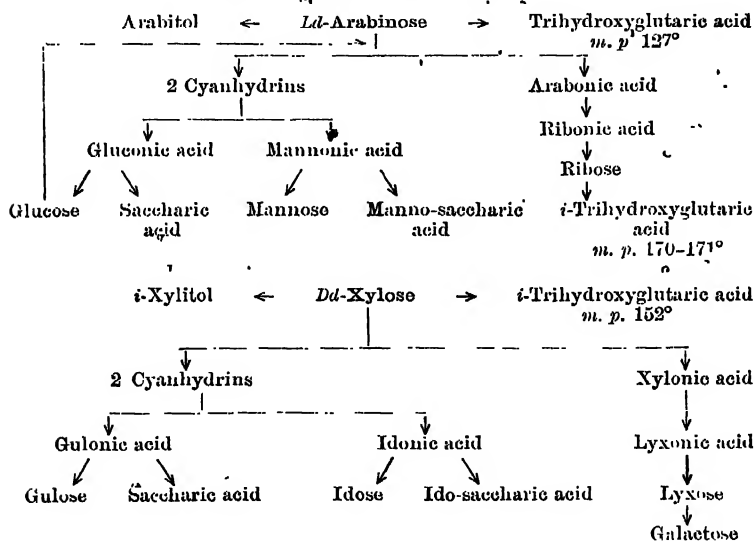
The following tables represent the synthesis of the higher from the lower sugars by Fischer's cyanhydrin method.



The sugars obtained by the degradation methods of Wohl and Ruff are represented as follows:



**Configuration of the Aldo-Hexoses.**<sup>1</sup> Before attempting to ascertain the space configuration of the large number of sugars which have been mentioned, it will be necessary to consider carefully the relation in which they stand to one another. This mutual relationship will be readily understood by reference to the tables given below. In the one the starting-point is arabinose, which is known in both laevo and dextro forms. From the laevo compound a set of *L*-derivatives would result, whilst the dextro compound would produce *D*-derivatives. In the second scheme xylose forms the starting-point. In both cases the natural products alone have been employed.



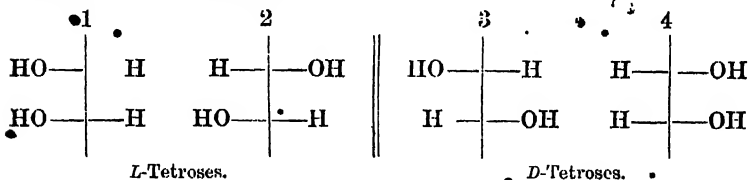
If it were possible to prepare glycollic aldehyde in quantity and build up the sugars in successive stages from it, by means of the cyanhydrin reaction, all the stereoisomers would probably be obtained and their configuration could be determined without difficulty. Let us attempt an imaginary scheme of this kind, adopting Meyer and Jacobson's plan of representing the space arrangement of the isomeric aldoses by projection formulae. If we further assume that the aldehyde group is always at the top and the primary carbinol group at the bottom, we can omit these two groups and merely represent the asymmetric carbon atoms with their hydroxyl and hydrogen appendages, the formulae being further simplified by denoting the asymmetric carbon by cross-lines.

<sup>1</sup> E. Fischer, *Ber.*, 1894, 27, 3208; *Lehrbuch der Stereochemie*, p. 90, by A. Werner.

Starting with glycollic aldehyde and converting this, by the imaginary process referred to, into the next higher sugar, two stereoisomeric trioses will be obtained, since one asymmetric carbon is present, which may be denoted thus:<sup>1</sup>



These will represent the two active glyceroses and form together an inactive or racemic combination. If the hydroxyl on the left of the asymmetric carbon represents the *L* and that on the right, the *D* configuration, the monosaccharoses derived from *L*-glycerose will belong to the *L* family and those from *D*-glycerose to the *D* family.<sup>2</sup> Each triose will yield two derivatives forming four tetroses, according to the general formula 2<sup>*n*</sup> where *n* is the number of asymmetric carbon atoms. They may be represented by adding on OH and H in the same and in the inverse order above the first two groups in the trioses.

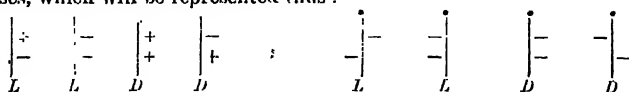


These four stereoisomers form two pairs of optical enantiomorphs,

<sup>1</sup> Some chemists prefer to adopt a form of shorthand proposed by Fischer (Ber., 1894, 27, 3189) to denote the position of the hydroxyl on the right or left by + or -. This system has not necessarily any reference to the optical character of the substance. Another system advocated by Rosanoff (J. Amer. Chem. Soc., 1906, 28, 314) is to indicate the aldehyde group by a dot and the position of the hydroxyl by a horizontal stroke projected on one side or other of the vertical line. The aldehyde group being on the top, *L*- and *D*-glycerose will appear in the two kinds of notation as follows:



By adding on a new asymmetric carbon above this, each glycerose will give two tetroses, which will be represented thus:



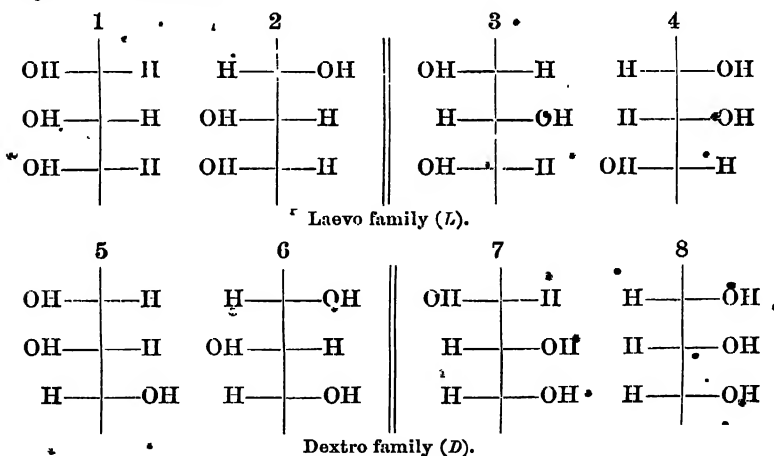
<sup>2</sup> It necessarily follows that the *L* family will be distinguished from the *D* family by the position of the lowest asymmetric group, the hydroxyl lying to the left in the *L* family and to the right in the *D* family, whatever positions the other groups may take.



namely, 1, 4 and 2; 3. Suppose now that the end groups in these compounds, instead of being different, are made identical, either by reduction to the corresponding alcohol, or by oxidation to the dibasic acid, the stereoisomers 1 and 4 become identical and represent the meso variety. This is easily seen by revolving one of the two through  $180^\circ$  in the plane of the paper. The number of stereoisomers is now reduced to three according to the formula,

$$2^{\frac{n}{2}-1} (2^{\frac{n}{2}} + 1)$$

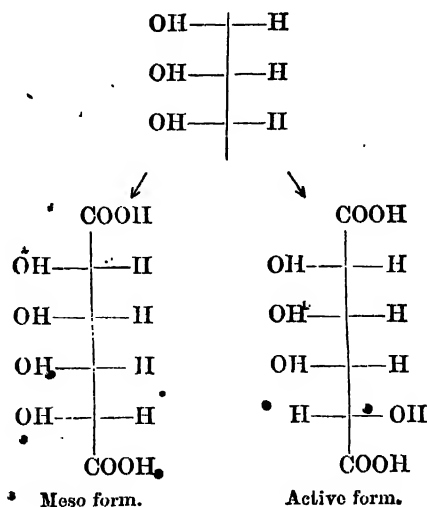
which is the general expression for the number of stereoisomers in a symmetrical molecule containing an even number of asymmetric carbon atoms. Thus, there are three tartaric acids and three erythritols, two being active enantiomorphs and the other an inactive meso compound. All these substances are known and are given in the table on p. 16. It follows from what has been said that *D*- and *L*-erythrose correspond to configurations 1 and 4, since they yield *i*-erythritol and *i*-tartaric acid on reduction and oxidation, whilst *D*-threose corresponds to the configuration 2 or 3. Since it is impossible to ascertain which of the two configurations represents the actual grouping in *D*-threose, it is customary to make an arbitrary choice in the case of *d*- and *l*-glucose and to derive all the other configurations from them. We must therefore first ascertain the configuration of these sugars. Continuing the process of imaginary synthesis, the four tetroses will each yield a pair of pentoses, making eight stereoisomers:



The optical enantiomorphs will be 1, 8; 2, 7; 3, 6; 4, 5. We

will now follow the same line of inquiry pursued in the case of the tetroses and suppose the end groups (by reduction to pentitols or by oxidation to trihydroxyglutaric acids) to become identical. The number of stereoisomers is now reduced to four; for  $1 = 8$ ;  $2 = 4$ ;  $3 = 6$ ;  $5 = 7$ . Which of these four pairs are active and which meso? It is now evident that with two similar end groups the middle carbon atom is no longer asymmetric in the usual meaning of the term. The activity is therefore determined by the two outer asymmetric carbon atoms. It follows that 1, 8 and 3, 6 are meso, and 2, 7 and 4, 5 are active forms when the end carbon groups are the same. We are now in a position to assign configurations to the six pentoses which have been described.

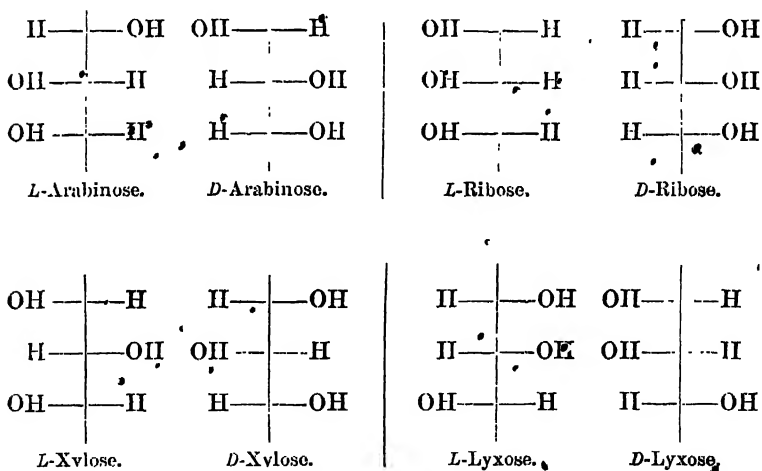
If we refer to the tables on p. 24, we notice that two of the pentoses, *D*-xylose and *L*-ribose, give inactive trihydroxyglutaric acids on oxidation. They will therefore be represented by one from each pair of the configurations 1, 8 and 3, 6. But *D*-xylose has been converted into two active saccharic acids, *L*-saccharic and *D*-ido-saccharic acid (see table, p. 24). The configuration of *D*-xylose cannot therefore be represented by 1, 8, since either enantiomorph would yield one inactive, internally compensated saccharic acid.



Consequently *D*- and *L*-xylose will be 3, 6 and *D*- and *L*-ribose will be 1, 8. The same process of reasoning may be applied to arabinose

and lyxose, which give active dibasic acids, and therefore have configurations 2, 7 and 3, 6. It can be shown that one of the pair of saccharic acids derived from the 4, 5 configuration is a meso-acid and cannot therefore represent arabinose, which gives two active saccharic acids. As the riboses are obtained from *D*- and *L*-arabinose by 'epimeric' change, *D*- and *L*-arabinose will have the configurations 2, 7. Lyxose, which is derived in the same way from xylose, will represent the fourth pair, 4, 5. As both *L*-arabinose and *L*-xylose are directly connected with *L*-glucose, the arbitrary configuration assigned to the latter (p. 26) will determine that of the two former as well as of all the other pentoses.

The configurations of the pentoses will stand as follows:



Each pentose will furnish two hexoses, making altogether sixteen stereoisomers. They may for convenience be divided into two groups of eight, known respectively as the mannitol and dulcitol group. In appending the names in the following table, we have anticipated the discussion of their configuration with the object of economizing space. The names of the dibasic acids are placed below the sugar or sugars from which they are derived for purposes of reference. It will be noticed that in the mannitol group the middle pair of asymmetric groups are diagonally situated; in the dulcitol group they are symmetrically arranged.

## Mannitol Group.

1	2	3	4
$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H}  \end{array}  $	$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH}  \end{array}  $	$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H}  \end{array}  $	$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH}  \end{array}  $
<i>L</i> -Mannose	<i>D</i> -Mannose	<i>L</i> -Idose	<i>D</i> -Idose
<i>L</i> -Manno-saccharic acid	<i>D</i> -Manno-saccharic acid	<i>L</i> -Ido-saccharic acid	<i>D</i> -Ido-saccharic acid
5	6	7	8
$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H}  \end{array}  $	$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH}  \end{array}  $	$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH}  \end{array}  $	$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH}  \end{array}  $
<i>L</i> -Glucose	<i>D</i> -Glucose <sup>1</sup>	<i>D</i> -Glucose	<i>L</i> -Glucose
<i>L</i> -Saccharic acid		<i>D</i> -Saccharic acid	

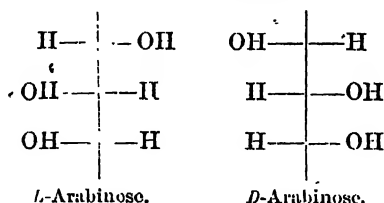
## Dulcitol Group.

9	10	11	12
$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H}  \end{array}  $	$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{OH} - \text{H} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH}  \end{array}  $	$  \begin{array}{c}  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H} \\    \\  \text{OH} - \text{H}  \end{array}  $	$  \begin{array}{c}  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH} \\    \\  \text{H} - \text{OH}  \end{array}  $
<i>L</i> -Galactose	<i>D</i> -Galactose	<i>L</i> -Allose (unknown)	<i>D</i> -Allose
<i>L</i> -Mucic acid		<i>D</i> -Allomucic acid	

<sup>1</sup> Fischer has attached the guloses to the glucoses from which they are derived by inversion (p. 13), seeing that each gulose is converted on oxidation into the same saccharic acid as that obtained from the parent glucose. Thus the gulose derived from *d*-glucose by inversion, and which gives *d*-saccharic acid on oxidation, is termed *d*-gulose. Rosanoff (*J. Amer. Chem. Soc.*, 1906, 28, 114) has, however, made it quite clear that neither fact is a criterion of family relationship, and has shown that *d*-gulose belongs to the *L*-family and *l*-gulose to the *D*-family, which latter also determines the sign of the two related sugars, namely, natural or *D*-xylose and *D*-idose (p. 24).

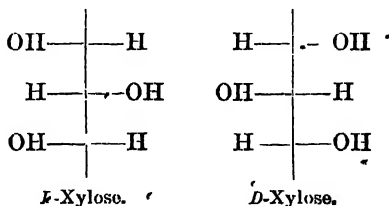
13		14		15		16	
H—	OH	H—	OH	OH—	H	OH—	H
H—	OH	OH—	H	OH—	H	H—	OH
H—	OH	OH—	H	OH—	H	H—	OH
OH—	H	OH—	H	H—	OH	H—	OH
<i>L</i> -Talose		<i>L</i> -Altrose (unknown)		<i>D</i> -Talose		<i>D</i> Altrose	
<i>L</i> -Talonic acid				<i>D</i> Talonic acid			

The configurations of the pentoses furnish a basis for that of the hexoses. As *D*- and *L*-glucose can be converted by Wohl's method into *D*- and *L*-arabinose, the configurations of the three lower asymmetric carbon atoms of the glucoses is given.

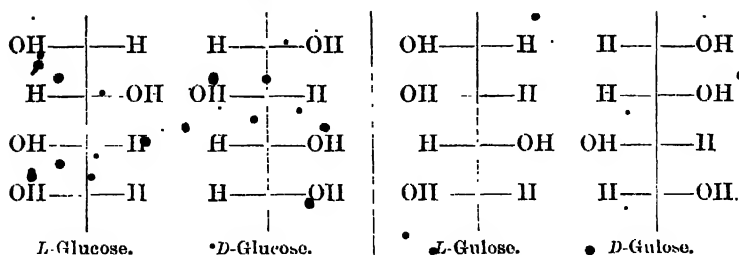


The same partial structure must be assigned to the mannoses, seeing that *L*-arabinose has been transformed into a mixture of *L*-mannose and *L*-glucose (p. 21).

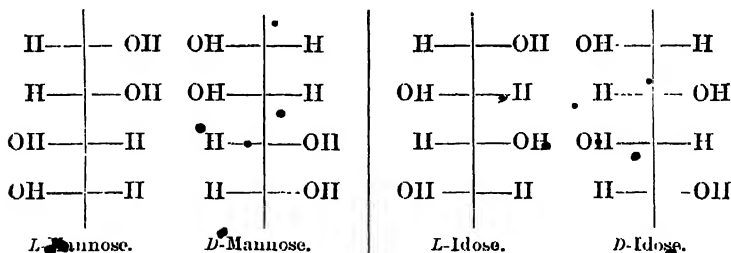
For similar reasons the idoses and guloses are related to *D*- and *L*-xylose and consequently contain the grouping :



Now, as the guloses are obtained from the glucoses by inversion of aldehyde and primary carbinol groups, the three upper asymmetric groups in gulose will represent the three lower asymmetric groups in glucose. Consequently, by combining the xylose and arabinose formulæ we obtain the glucoses and the guloses.

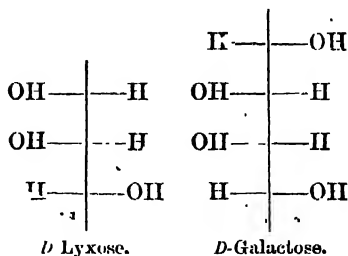


The second formula is arbitrarily assigned to *D*-glucose and the others consequently follow. The configuration of the three lower carbon atoms will also enable similar distinctions to be drawn between the *D*- and *L*-enantiomorphs of arabinose and xylose. As both *L*-glucose and *L*-mannose are obtained by the cyanhydrin synthesis from *L*-arabinose, as moreover they both yield the same osazone, and as mannonic acid is partly converted into gluconic acid by inversion, the only difference in their configurations must relate to the top asymmetric carbon atom. The same difference exists between the idoses and guloses. The mannoses and idoses will consequently have the following structure:



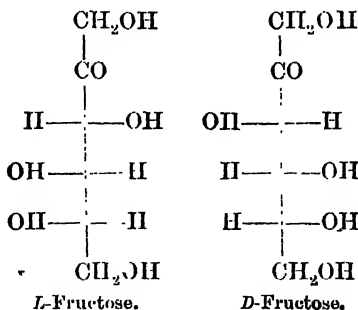
The above configurations are confirmed by the following observations. It will be seen on reference to the eight configurations of the mannitol group (p. 29) that, whilst each of the first four should yield a different saccharic acid on oxidation, the second four require only one saccharic acid for each pair of stereoisomers, and this strictly theoretical deduction stands in complete harmony with the facts. The configurations of the galactoses and taloses are determined as follows: as the galactoses give on oxidation the same inactive, or meso-mucic acid, their configuration is limited to the first two pairs of stereoisomers (9-12) of the dulcitol group. But *D*-galactose has been transformed into *D*-lyxose, and the latter can only differ from xylose in the configurations of the top asymmetric group.

Consequently lyxose and galactose will have the following configurations :

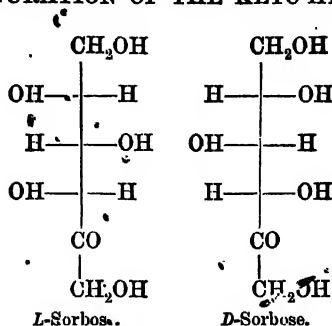


As *D*- and *L*-talose are obtained by epimeric change from *D*- and *L*-galactose, the configuration of these two members is known. Finally, the configuration of *D*-allose and *D*-altrose can be ascertained from the fact that they are built up from *D*-ribose and that *D*-allose gives allcinic acid on oxidation. *D*-Allose is therefore represented by 12 and *D*-altrose by 16.

**Configuration of the Keto-Hexoses.** The configuration of the aldo-hexoses being known, that of the keto-hexoses is readily ascertained. Thus *D*-glucose has been transformed into *D*-fructose, and they both yield the same glucosazone. It follows that the three asymmetric groups of *D*-fructose and *D*-glucose are identical.

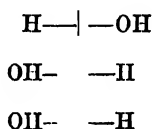


As *D*-sorbitol is obtained by oxidising *D*-sorbitol, which is the alcohol corresponding to *D*-glucose, the configuration of the three lower asymmetric groups in *D*-sorbitol must be that of *D*-glucose.

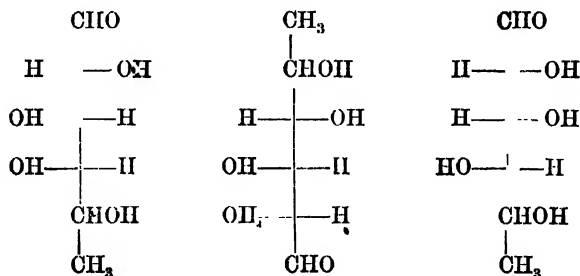


This is confirmed by the identity of the osazone of *L*-sorbos ( $\psi$ -tagatose) with those of *L*-gulose and *L*-idose.

**Configuration of the Methyl Pentoses group.** *L*-Rhamnose gives, on oxidation, the same *L*-trihydroxyglutaric acid as that derived from *L*-arabinose, and consequently must contain the complex:



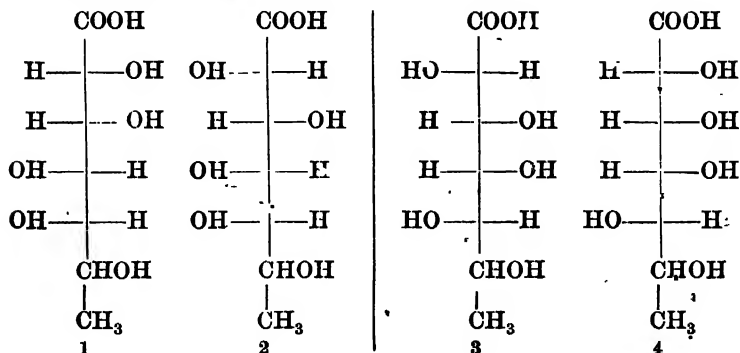
*L*-Rhamnose is therefore represented by one of the following forms, on the assumption that the methyl group is removed, and the remaining two end groups converted into carboxyl:



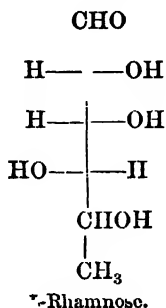
Now *L*-rhamnose yields by the cyanhydrin reaction two,  $\alpha$ - and  $\beta$ -, rhamnohexonic acids, one of which gives mucic and the other *L*-talo-mucic acid on oxidation. The rhamnohexonic acids will consequently be represented by two of the following pairs of configurations, one



being derived from the first and the other from the second of the above formulae for rhamnose :



It is obvious that the rhamnohexonic acids cannot be represented by 1 and 2, since the configuration of these two compounds belongs to members of the mannitol group which are already known. Therefore, as mucic acid is an inactive meso compound, the rhamnohexonic acid corresponding will be represented by 3, whilst the second rhamnohexonic acid which gives *L*-talomucic acid has the configuration 4. Rhamnose is therefore represented by the first of the two configurations given above, the proof of which incidentally determines that of galactose and *L*-talose (which correspond to mucic and *L*-talomucic acid) and partially that of the other rhamnose derivatives.



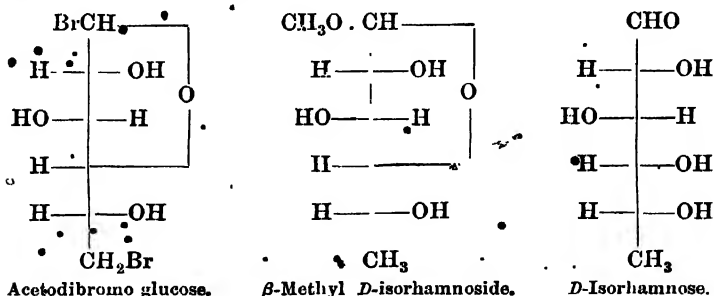
The complete configuration has been ascertained through that of *D*- and *L*-isorhamnose. *L*-Isorhamnose (*epi*-rhamnose) was obtained by Fischer and Herborn<sup>1</sup> by the epimeric conversion of *L*-rhamnose, and the *D*-enantiomorph by Votoček from purgic acid.<sup>2</sup> Now Fischer

<sup>1</sup> Ber., 1896, 29, 1961.

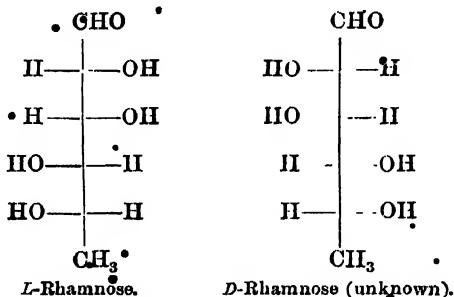
<sup>2</sup> Ber., 1911, 44, 819, 3287.

# CONFIGURATION OF THE METHYL PENTOSSES GROUP. • 35

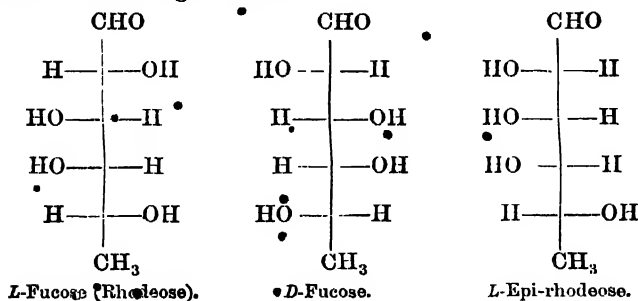
and Zach<sup>1</sup> have shown that aceto dibromoglucose (p. 45) can be converted by zinc dust and acetic acid through  $\beta$ -methyl *D*-isq-rhamnoside into *D*-isorhamnose.



It follows, therefore, that *L*- and *D*-rhamnose have the following configuration :



*D*-fucose gives, on oxidation, the same trihydroxyglutaric acid as *D*-arabinose, but as fucohexonic acid, prepared by the hydrogen cyanide synthesis, gives no mucic acid on oxidation, the fucoses must possess the following formulae<sup>2</sup>:

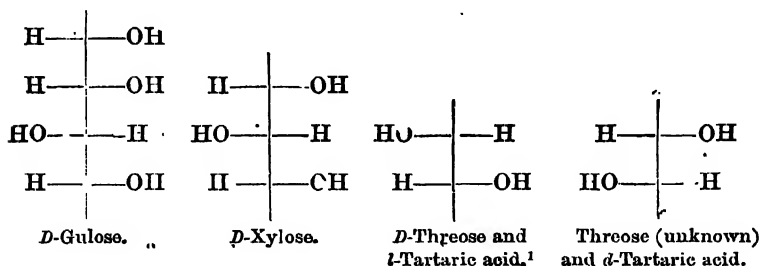


<sup>1</sup> Ber., 1912, 48, 3761.

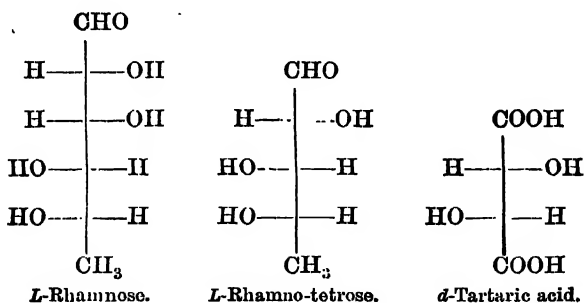
<sup>2</sup> Mayer and Tollens, Ber., 1907, 40, 2434.

*L*-Epi-rhodoose is prepared by epimeric change from *L*-fucose. The *D* compound is unknown.

**Relative Configuration of the Tartaric Acids.** The relation of the *d*- and *l*-tartaric acids to the glucoses can be readily ascertained through *D*-threose, which, on the one hand, gives *l*-tartaric acid by oxidation, and, on the other, is derived from *D*-xylose and *D*-gulose (p. 16).



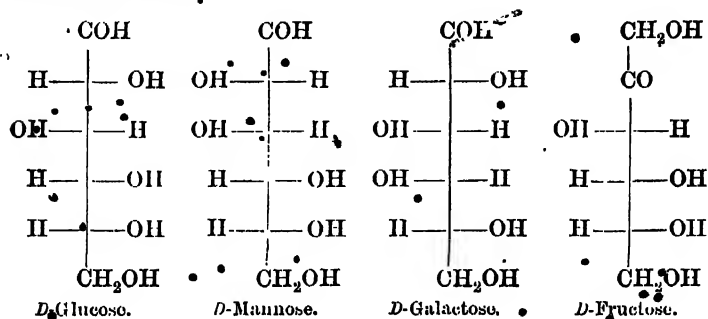
The configuration of *d*-tartaric acid may also be derived from that of rhamnose, since Fischer has shown that rhamnose may be converted by Wohl's method into methyl tetrose, which yields *d*-tartaric acid on oxidation.



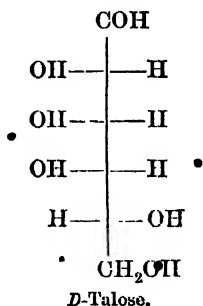
**Fermentation of the Monosaccharoses.** Pasteur was the first to show that a solution of racemic acid becomes laevo-rotatory in presence of *penicillium*, owing to the destruction of the dextro-tartaric acid by the fungus—an observation which has been frequently utilized in the attempt to isolate one of the optical enantiomorphs from an inactive mixture (Part II, p. 181). Fischer has shown that this selective action is exhibited in a very marked degree by the beer yeasts in producing fermentation of carbohydrates. Of the twelve known aldo-hexoses only the three natural sugars are fermentable.

<sup>1</sup> Wohl and Momba, *Ber.*, 1917, 50, 455.

viz., *D*-glucose, *D*-mannose, and *D*-galactose, and of the keto-hexoses only *D*-fructose is decomposed. All the yeasts susceptible of inducing fermentation transform *D*-glucose, *D*-mannose, and *D*-fructose, with almost equal velocity, but the action of yeast on *D*-galactose is slower, and certain species—*saccharomyces apiculatus*, and *pyoductivus*—are totally without action upon it.<sup>1</sup> A comparison of the configuration of these four sugars exhibits the differences of molecular grouping:



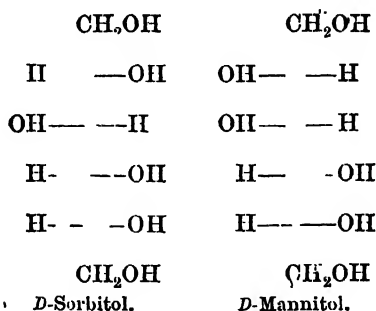
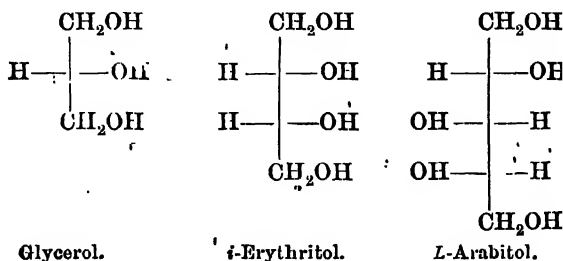
In glucose, mannose, and fructose, the grouping of the H and OH round the three lower asymmetric carbon atoms is the same, but differs from that in galactose, a fact which may account for the slow fermentative action of the latter. The other hexoses are not fermentable. The small difference in configuration which suffices to arrest the action is seen in the case of *D*-talose, which only differs from *D*-galactose by the position of one hydroxyl group.



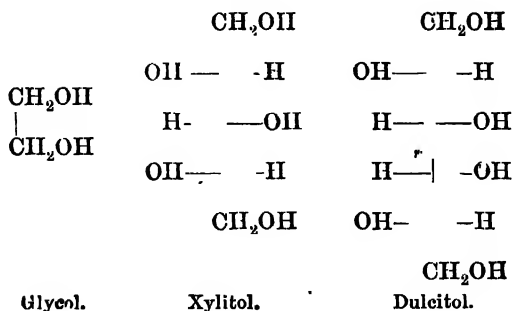
Of the other monosaccharoses only glycerose, dihydroxyacetone, and manno-nonose, that is, sugars with three, or a multiple of three, carbon atoms are known to undergo fermentation. This curious

<sup>1</sup> Sclator, *Trans. Chem. Soc.*, 1906, 89, 128; 1908, 93, 217.

selective action of the organism is repeated in the case of the polyhydric alcohols. Bertrand,<sup>1</sup> in his brilliant investigation on the sorbose bacterium, has shown that the conversion of the alcohols into ketoses is dependent on their configuration, and that whereas glycerol, *i*-erythritol, *L*-arabitol, *D*-sorbitol, *D*-mannitol, &c., with the following configurations, are oxidised by the bacterium, . . .

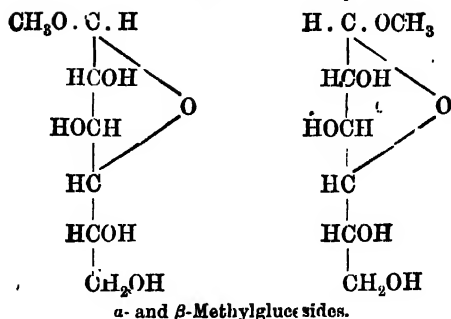


glycol, xylitol, and dulcitol are not.



<sup>1</sup> *Ann. Chim. Phys.*, 1904 (8), 5, 181.





The following is a list of aldoses and ketoses obtained in this way:

*Aldosides.*

$\alpha$ -Methyl *d*-glucoside  
 $\beta$ -Methyl *d*-glucoside  
 $\alpha$ -Methyl *l*-glucoside  
 $\beta$ -Methyl *l*-glucoside  
 $\alpha$ -Methyl *dl*-glucoside  
 $\alpha$ -Ethyl *d*-glucoside  
 Propyl *d*-glucoside  
 Phenyl *d*-glucoside  
 Benzyl *d*-glucoside  
 $\alpha$ -Methyl *d*-galactoside  
 $\beta$ -Methyl *d*-galactoside  
 Ethyl *d*-galactoside

Methyl *d*-mannoside  
 Methyl *l*-mannoside  
 Methyl rhamnoside  
 Ethyl rhamnoside  
 Methyl arabinoside  
 Ethyl arabinoside  
 Benzyl arabinoside  
 $\alpha$ -Methyl xyloside  
 $\beta$ -Methyl xyloside  
 Methyl gluco-heptoside

*Ketosides.*

Methyl sorboside  
 Methyl fructoside

What has been said of the selective action of yeast and the sorbose bacterium applies to that of enzymes on the artificial alkyl glucosides. 'Fischer' made the interesting observation that an aqueous extract of pulverized yeast cells, which contains an enzyme *maltase*, hydrolyses  $\alpha$ -methyl *d*-glucoside, but has not the least action on the  $\beta$ -methyl *d*-glucoside. Exactly the reverse happens with the emulsin of bitter almonds which hydrolyses the  $\beta$ -glucoside, whilst the  $\alpha$  modification remains unchanged. The ethyl, and phenyl glucosides, of each of which only one modification is known, behave like the  $\alpha$ -methyl compound, and probably belong to the same category. Similar differences have been observed in the case of other glucosides both natural and artificial; in other cases again neither enzyme has any action.

The following is a list of natural and artificial glucosides. The

<sup>1</sup> *Zeit. physiol. Chem.*, 1898, 26, 61.

action of the enzyme is denoted by + when it produces hydrolysis and by - when it is without action.

Artificial glucosides.	Emulsin.	Maltase.
$\alpha$ -methyl <i>d</i> -glucoside	-	+
$\beta$ -methyl <i>d</i> -glucoside	+	-
$\alpha$ -methyl <i>l</i> -glucoside	-	-
$\beta$ -methyl <i>l</i> -glucoside	-	-
$\alpha$ -ethyl <i>d</i> -glucoside	-	+
$\beta$ -ethyl <i>d</i> -glucoside	+	-
phenyl <i>d</i> -glucoside	+	-
$\alpha$ -methyl <i>d</i> -galactoside	-	-
$\beta$ -methyl <i>d</i> -galactoside	-	-
$\beta$ -methyl <i>d</i> -mannoside	-	-
$\beta$ -methyl <i>l</i> -mannoside	-	-
methyl <i>l</i> -arabioside	-	-
$\alpha$ -methyl <i>l</i> -xyloside	-	-
$\beta$ -methyl <i>l</i> -xyloside	-	-
methyl rhamnoside	-	-
methyl gluco-heptoside	-	-
methyl sorboside	-	-
methyl fructoside	-	+

Natural glucosides.	Emulsin.	Maltase.
Salicin	+	-
Helicin	+	-
Aesculin	+	-
Coniferin	+	-
Phillyrin	-	-
Aprin	-	-
Syringin	+	-
Saponin	-	-
Phloridzin	-	-
Mandelo nitrile glucoside	+	-
Amygdalin	+	-
Quercitrin	-	-

It would appear that the majority of the natural glucosides belong to the group of  $\beta$ -glucosides.

We shall see presently that the disaccharoses are subject to the same selective hydrolysis by enzymes and are divisible into two stereochemically related groups.

It should be noted that this action of the enzyme is reversible, for Bayliss and Bourquelot and Bridel and others have shown that  $\alpha$ - and  $\beta$ -alkyl glucosides of mono- and polyhydric alcohols may be synthesised by introducing into a mixture of the sugar and alcohol one or other of the two enzymes (see p. 99).<sup>1</sup>

<sup>1</sup> Bourquelot, *Journ. Pharm. Chim.*, 1914, 10, 361; see also plant synthesis of salicin, Ciamician and Raveana, *R. Accad. Lincei*, 1909, 18, i, 419.



**Structure of Glucose as determined by Enzyme Action.** The above observations on enzyme action will enable us to understand a new theory of the structure of glucose and the other monosaccharoses which has been incidentally referred to on p. 3. It is well known that many of the sugars are subject to mutarotation (Part II, p. 232), that is, to a change in rotation when the freshly prepared solution of the substance is allowed to stand, or, more quickly, if a trace of alkali is added and the liquid warmed. Thus, *d*-glucose freshly dissolved in water exhibits a rotation of  $[\alpha]_D = +110^\circ$ , which becomes constant when it has dropped to about half, i.e.  $[\alpha]_D = +52.5^\circ$ . The change has been variously ascribed to hydration and to change of structure. Both the modifications known as  $\alpha$ - and  $\beta$ -glucose and an additional  $\gamma$ -glucose having a rotation  $[\alpha]_D = +20^\circ$  have been isolated by Tanret.<sup>1</sup> The first was prepared by crystallization from water or dilute alcohol at the ordinary temperature, the second by precipitation of a concentrated aqueous solution with alcohol, and the last by crystallizing its aqueous solution above  $98^\circ$ . Similar modifications of rhamnose, galactose, arabinose, and lactose were prepared. Whichever of the three modifications is dissolved, the rotation becomes constant when  $[\alpha]_D = +52.5^\circ$ . It appeared, therefore, not improbable that the intermediate  $\beta$ -glucose represented a mixture of dynamic isomers. Simon<sup>2</sup> was the first to suggest that  $\alpha$ - and  $\gamma$ -glucose correspond to  $\alpha$ - and  $\beta$ -methyl glucoside, since the mean values of the rotation are nearly the same:

$\alpha$ -methyl glucoside	+ 157°	$\alpha$ -glucose	+ 110°
$\beta$ - " "	- 32	$\gamma$ - " "	+ 20
	+ 125		+ 130
mean = + 62.25		mean = + 65	

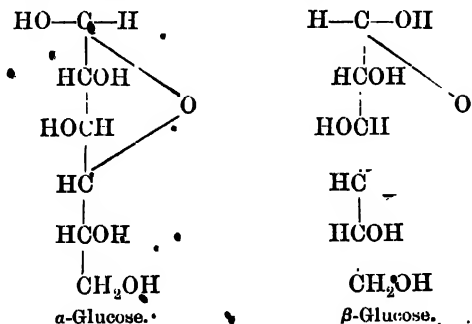
If this is the case,  $\alpha$ -methyl glucoside, on hydrolysis, should yield a sugar of high,  $\beta$ -methyl glucoside of low rotatory power. The glucoses themselves are, however, so sensitive to ordinary chemical reagents that hydrolysis of the glucosides by acids or alkalis is precluded. E. F. Armstrong<sup>3</sup> hit upon the ingenious device of hydrolysing the glucosides by the aid of the enzymes, emulsin and maltase, and determining the rise or fall of rotation when equilibrium was reached, a process which can be quickly effected by adding a trace of alkali to the resulting solution. Small but definite indications of the existence of two glucoses were obtained, which must consequently

<sup>1</sup> *Compt. rend.*, 1895, 120, 1060.

<sup>2</sup> *Compt. rend.*, 1901, 132, 487.

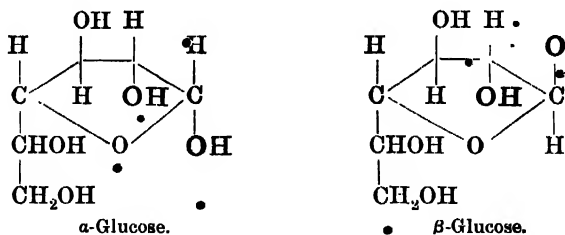
<sup>3</sup> *Trans. Chem. Soc.*, 1906, 85, 1306.

be represented by configurations similar to the  $\alpha$ - and  $\beta$ -glucosides and named to correspond,  $\alpha$ - and  $\beta$ -glucose.<sup>1</sup>



The lactone structure of the glucoses is supported by many independent facts, notably the evidence derived from observations of the magnetic rotation,<sup>2</sup> the rotational value (p. 42), and the electrical conductivity of boric acid in presence of the sugar.

By the latter method Bolseken<sup>3</sup> has shown that the electrical conductivity of boric acid is increased when the hydroxyl groups lie on the same side of the molecule—in this case, on the same side of the lactone ring. The higher conductivity in presence of  $\alpha$ -glucose corresponds, therefore, to the formula given above. The two formulae are reproduced in a somewhat modified form in order to emphasize the distinction.



Another significant fact pointed out by Hudson<sup>4</sup> is that as the  $\alpha$  and  $\beta$  forms only differ in configuration in respect of the end carbon group the sum or difference in molecular rotations of each pair should be a constant quantity, and this will be found to be the case. The hexose sugars, for example, in the table on p. 51 show a nearly constant difference.

<sup>1</sup> C. S. Hudson, *J. Amer. Chem. Soc.*, 1909, 31, 66; 1917, 39, 320.

<sup>2</sup> Perkin, *Trans. Chem. Soc.*, 1903, 81, 177.

<sup>3</sup> Ber., 1898, 46, 2612.

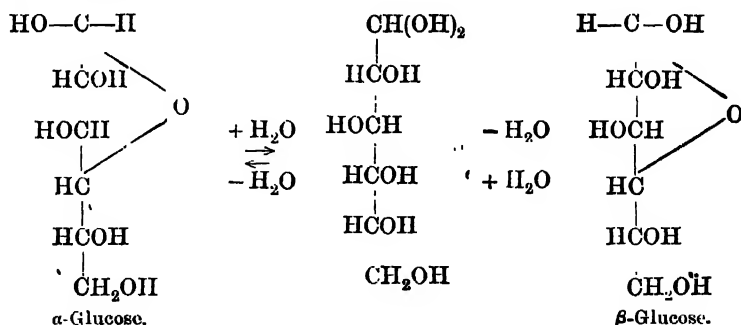
<sup>4</sup> *J. Amer. Chem. Soc.*, 1909, 31, 66.

As most of the sugars exhibit mutarotation and exist in isomeric forms, the lactone structure is now generally adopted. The following table gives the rotations of the  $\alpha$  and  $\beta$  forms and the equilibrium mixture:<sup>1</sup>

Carbohydrate.	$\alpha$ -Form.	$\beta$ -Form.	Equilibrium mixture.
<i>d</i> -Glucose	+ 110°	+ 20°	+ 52.5°
<i>d</i> -Mannose	+ 76°*	- 14°	+ 14°
<i>d</i> -Galactose	+ 140°	+ 58°	+ 81°
<i>d</i> -Fructose	+ 17°	- 140°	- 93°
<i>l</i> -Arabinose	+ 76°	+ 184°	+ 104°
<i>d</i> -Xylose	+ 100°	- 8°*	+ 19°
<i>l</i> -Rhamnose	- 7°	+ 32°	+ 3°
<i>d</i> -Maltose	+ 166°*	+ 119°	+ 137°
<i>d</i> -Lactose hydrate	+ 86° <sup>A</sup>	+ 85°	+ 55.8°
<i>d</i> -Melibiose	+ 171°	+ 124°	+ 143°

Calculated values.

Lowry<sup>2</sup> has discussed at some length the mechanism of the isomeric change by which the two sugars are formed, which he regards as a reversible process effected by the successive addition and removal of water, thus:

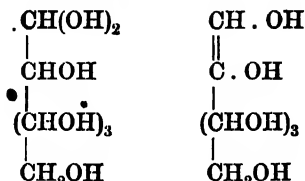


The existence of the above intermediate compound may also explain the interconversion, under the influence of alkalis, of glucose, mannose, and fructose, and similar changes, observed by Lobry de Bruyn and van Ekenstein, and already referred to on p. 13. Wphl<sup>3</sup> has suggested that the compounds in question may have a common enolic form derived from the intermediate product by loss of water, thus:

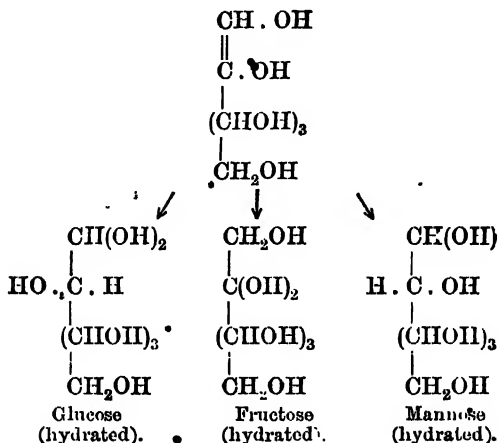
<sup>1</sup> Hudson, *J. Amer. Chem. Soc.*, 1910, 32, 889; 1917, 39, 1013.

<sup>2</sup> *Trans. Chem. Soc.*, 1903, 85, 1314.

<sup>3</sup> *Ber.*, 1900, 33, 8093.



The production of glucose, mannose, and fructose can be represented as a hydration process followed by lactone or ketone formation thus:

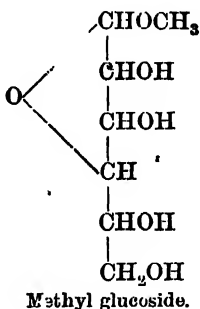
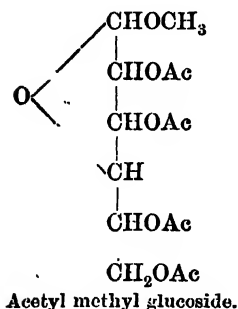
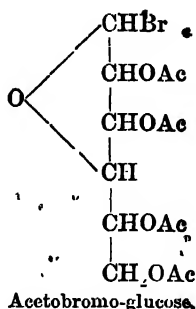
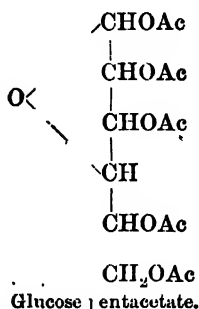


In addition to  $\alpha$ - and  $\beta$ -methyl glucoside, there are many other derivatives of  $\alpha$ - and  $\beta$ -glucose. It has long been known that a different glucose acetate is formed by the use of acetic anhydride and zinc chloride, or acetic anhydride and sodium acetate, according to the method of preparation.<sup>1</sup> These have now been brought into relation with the two methyl glucosides by a method devised by Königs, and successfully applied by Fischer and Armstrong.<sup>2</sup> It consists in converting the pentacetates of glucose by means of liquid hydrogen chloride or bromide or, more easily, by the action of a saturated solution of these two acids in acetic acid, into the  $\alpha$ - and  $\beta$ -acetochloro- and acetobromo-glucoses. The halogen in the latter is then replaced by methoxyl by the combined action of methyl

<sup>1</sup> Erwig and Königs, *Ber.*, 1889, 22, 1464; Franchimont, *Rec. trav. Pays-Pas*, 1892, 11, 106.

<sup>2</sup> *Ber.*, 1901, 34, 2885.

iodide and silver carbonate, and the acetyl methyl glucosides are then hydrolysed to remove the acetyl groups.



In this way the pentacetate obtained by the action of acetic anhydride in presence of zinc chloride, or when  $\alpha$ -glucose is acetylated in presence of pyridine,<sup>1</sup> was shown to be the  $\alpha$ -compound, whilst that obtained with acetic anhydride and sodium acetate, or with  $\beta$ -glucose in presence of pyridine, was mainly the  $\beta$ -compound.

Pentacetates of other hexoses and octacetates of the disaccharoses have also been prepared in isomeric forms.<sup>2</sup>

In addition to the isomeric forms of glucose pentacetate, acetochloro- and acetobromo-glucose, acetomethyl glucoside, and methyl glucoside, the following glucose derivatives are known:  $\alpha$ - and  $\beta$ -acetonitro glucose,  $\alpha$ - and  $\beta$ -tetracetyl glucose (obtained from acetobromo glucose by shaking the ether solution with silver carbonate

<sup>1</sup> Behrend, *Annalen*, 1904, 231, 369; 1907, 353, 109.

<sup>2</sup> Hudson, *J. Amer. Chem. Soc.*, 1915, 37, 1272, 1589; 1916, 38, 1223.

and a little water), and various methyl glucoses and methyl glucosides, which are referred to on p. 48.

Most of them are interconvertible and exhibit mutarotation yielding an equilibrium mixture of constant rotation. The following table gives the specific rotations of the two series :

Glucose derivative.	$\alpha$ -series $[\alpha]_D$ .	$\beta$ -series $[\alpha]_D$ .
Pentacetate	+ 100°	+ 3°
Acetochloro	—	+ 165
Acetobromo	—	+ 198
Acetonitro	+ 1.5	+ 149

Fischer and others have utilized acetobromo glucose and similar derivatives of other hexoses for obtaining a variety of glucosides. Acetobromo glucose in ether solution combines with a variety of alcohols in presence of silver carbonate;<sup>1</sup> also with phenols in presence of alkali, with morphine,<sup>2</sup> with the silver salts of thiouræthanes to form mustard-oil glucosides,<sup>3</sup> and with the silver compounds of some of the purine compounds (p.166), giving glucosides of these compounds, termed *nucleosides*.<sup>4</sup>

• As the imine, anilide, phenylhydrazone, and oxime of glucose either occur in two isomeric forms or exhibit mutarotation, it is probable that these compounds likewise possess the  $\alpha$ - and  $\beta$ -lactone structure.<sup>5</sup> It should be pointed out that Lowry's explanation of mutarotation, described on p. 44, presents an obvious difficulty when applied to such substances as glucosepentacetate and acetochloroglucose. An alternative explanation has been suggested by E. F. Armstrong,<sup>6</sup> in which the lactone oxygen is supposed to combine with water, forming an oxonium hydrate. The hydrogen of the terminal carbon is then removed with the hydroxyl of the oxonium hydrate group, which leaves the terminal carbon momentarily unsaturated. The residual hydrogen of the oxonium hydrate group then moves into one or other of the two free positions, giving  $\alpha$ - or  $\beta$ -compounds, a process which may be represented in skeleton form thus :

<sup>1</sup> *Ber.*, 1910, 43, 2521 ; 1912, 45, 2467 ; 1914, 47, 1377.

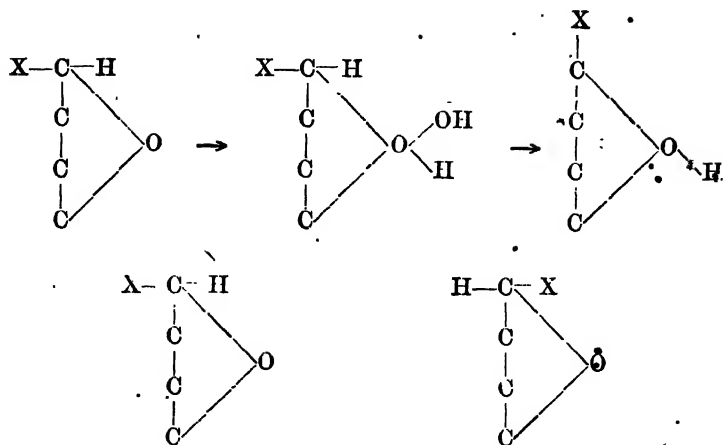
<sup>2</sup> Mannich, *Annalen*, 1912, 394, 223.

<sup>3</sup> Schneider and others, *Ber.*, 1914, 47, 1258, 2218.

<sup>4</sup> Fischer and Helferich, *Ber.*, 1914, 47, 210, 1058.

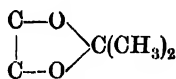
<sup>5</sup> Irvine and Morde, *Trans. Chem. Soc.*, 1908, 93, 96, 1429 ; 1913, 103, 238.

<sup>6</sup> *Journ. Physiol.*, 1905, 33, 17.

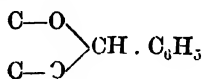


The view is, however, not easy to reconcile with the fact that mutarotation takes place in formamide solution, that is, in the absence of water.<sup>1</sup>

**The Methyl Glucoses.** Fischer<sup>2</sup> showed some years ago that the sugars combine with one or two molecules of acetone and also with benzaldehyde to form well-characterized acetone (isopropylidene) and benzylidene compounds containing the groups



Isopropylidene.



Benzylidene.

Later, Purdie and Irvine<sup>3</sup> devised a method for alkylating the alkyl glucosides, by the action of methyl iodide in presence of silver oxide, whereby they were able to obtain tri- and tetra-methyl  $\alpha$ - and  $\beta$ -methyl glucosides and, by subsequent hydrolysis, a variety of tri- and tetra-methyl hexoses and trimethyl pentoses. The method does not, however, admit of the isolation of the lower methylated derivatives. But by methylating benzylidene and mono- and di-acetone derivatives and subsequently hydrolysing the product (the benzylidene and isopropylidene groups are readily eliminated), not only was it possible to obtain simpler alkylated compounds, but to ascertain the position of the entrant alkyl groups.

<sup>1</sup> Mackenzie and Ghosh, *Abstr.*, 1915, ii. 301.

<sup>2</sup> *Ber.*, 1895, 28, 1145.

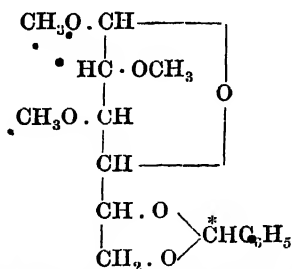
<sup>3</sup> *Trans. Chem. Soc.*, 1903, 83, 1021 ; 1904, 85, 1049 ; 1905 87, 900.

The following table is given by Irvine and Scott<sup>1</sup> by way of illustrating the application of the method to the preparation of the different stages of alkylation :

Condensation product.	Methoxyl groups added.	Methoxyl groups hydrolysed.	Methyl glucose.
Glucosyl diacetone	1	2 mols. acetone	Monomethyl glucose
Benzylidene $\alpha$ -methyl glucoside	2	Methyl alcohol and benzaldehyde	Dimethyl glucose
Glucose monoacetone	3	1 mol. acetone	Trimethyl glucose
Methyl glucoside	4	Methyl alcohol	Tetramethyl glucose

The structure of dimethyl glucose has been determined from the following considerations. As the sugar retains its reducing properties, the lactone groups (carbon groups 1 and 4)<sup>2</sup> need not be considered. There are, consequently, four remaining hydroxyl groups with any pair of which the benzaldehyde may combine. Now it appears that the disposition of the hydroxyl groups on the same side of the chain determines condensation with benzaldehyde, for, whereas methyl glucoside with only one such pair of disposable hydroxyl groups combines with one molecule of benzaldehyde, methyl mannoside with two pairs forms a dibenzylidene derivative.

The configuration of the dimethyl derivative of benzylidene dimethyl  $\alpha$ -methyl glucoside will then be the following:



**Benzylidene dimethyl  $\alpha$ -methyl glucoside.**

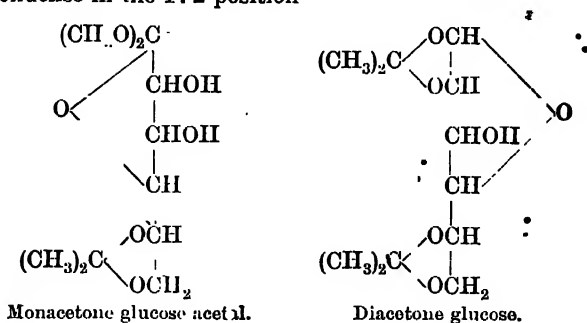
Incidentally it may be mentioned that the carbon atom with the asterisk is asymmetric and the condensation introduces a new asymmetric group. In accordance with the theory, two active compounds

<sup>1</sup> *Trans. Chem. Soc.*, 1913, 103, 566.

<sup>2</sup> To avoid confusion, which the present use of the Greek letters for indicating both stereo- and structural isomers entails, the carbon atoms in the chain are indicated by the numbers 1 to 6, beginning with the terminal aldehyde (or lactone) group.



have been isolated. Further, as monoacetone glucose acetal undergoes further condensation with acetone to form a diacetone glucose with elimination of the acetal group, the second acetone molecule must condense in the 1.2 position<sup>1</sup>



Consequently the methyl derivative obtained from the diacetone can only occupy the position 3.

These examples will serve to illustrate the method adopted by Irvine and his collaborators in studying the structure of the alkylated sugars. The following table gives the specific rotations of the  $\alpha$  and  $\beta$  series of methyl glucosides:<sup>2</sup>

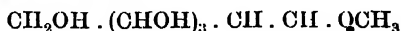
	$\alpha$	$\beta$
Monomethyl glucose	+96°	+32°
2.3 Dimethyl glucose	+82°	+6°
2.3.5.6 Tetramethyl glucose	+101°	+73.5°

**$\gamma$ -Methyl Glucoside.** In Macdonald's experiments on monoacetone glucose azetal, described above, it was found that when this substance is heated *in vacuo* it loses methyl alcohol, giving a methyl glucoside monoacetone, and by removal of acetone a new methyl glucoside, which, however, cannot be derived from either  $\alpha$ - or  $\beta$ -methyl gluco-

<sup>1</sup> Macdonald, *Trans. Chem. Soc.*, 1913, 103, 1896.

<sup>2</sup> Nef has put forward the view that the  $\alpha$  and  $\beta$  compounds represent a different lactone formation, the one being a 1.4 lactone and the other a 1.3 lactone (*Ber.*, 1914, 47, 1980). Fischer has pointed out that this view is quite untenable, seeing that not only do these substances undergo mutarotation, but that the glucose pentacetates give with HCl and HBr the same acetochloro- and acetobromo-glucose; that  $\beta$  acetobromo glucose and silver nitrate gives  $\beta$ -acetonitro glucose, which by recrystallization passes into the  $\alpha$  compound, and that the two tetramethyl methyl glucosides give on hydrolysis tetramethyl glucose which also shows mutarotation. In all these cases the substituted hydroxyl group in the 3 or 4 position must undergo an interchange of its substituent group from the one position to the other when the  $\alpha$  compound passes into the  $\beta$  or vice versa on mutarotation. That both substances are 1.4 lactones, upon the structure of which that of the methyl glucosides depends, follows from the configuration in its relation to the condensation products with benzaldehyde and acetone and to the rotational values which are discussed on p. 51.

side, seeing that they do not combine with acetone whereas the new methyl glucoside does.<sup>1</sup> The same product had been previously obtained by Fischer<sup>2</sup> by the partial hydrolysis of glucose dimethyl-acetal with hydrochloric acid. It is a syrupy liquid which can be distilled *in vacuo* without decomposition, but, unlike α- and β-methyl glucoside, is hydrolysed with the rapidity of cane-sugar and certain fructosides. It is also characterized by its rapid reduction of permanganate and by the ease with which it unites with acetone. From these and other considerations Irvine has provisionally assigned to it the formula of a 1.2 lactone.



γ-Methyl glucoside.

A tetramethyl γ-glucose was also prepared by methylation and subsequent hydrolysis. It seems not unlikely that one of the two isomeric fructose pentacetates (which are not interconvertible) is a derivative of γ-fructose.<sup>3</sup>

### Rotatory Power and Configuration of the Monosaccharoses.

A number of interesting computations have been made by Hudson<sup>4</sup> showing the relation of rotatory power to configuration, a relation which incidentally may be said to support the theory of optical superposition (see Part II, p.233). He points out that, as the difference in rotation of the α- and β-hexoses depends solely on the configuration of the terminal asymmetric group, then if *A* stands for this group and *B* for the remaining asymmetric carbons which are common to the two, the rotational value for the whole molecule will be *A* + *B* in the one stereoisomer and − *A* + *B* in the other. *B* will of course vary in the different sugars, but *A*, which depends solely on the end asymmetric group, should remain constant if uninfluenced by the rest of the molecule. This is given by the difference between the two rotations *A* + *B* − (− *A* + *B*) = 2*A*.

Sugar.	[M] <sub>D</sub>	Δ(2 <i>A</i> )	Sugar.	[M] <sub>D</sub>	Δ(2 <i>A</i> )
α- <i>D</i> -glucose	196°	160	α- <i>L</i> -arabinose	114°	− 162
β-       "	36		β-       "	276	
α- <i>D</i> -galactose	252	157	α- <i>D</i> -lactose	294	
β-       "	95		β-       "	120	174

<sup>1</sup> Irvine, Fyfe and Hogg, *Trans. Chem. Soc.*, 1915, 107, 524.

<sup>2</sup> *Ber.*, 1914, 47, 1980.

<sup>3</sup> Hudson and Brauns, *J. Amer. Chem. Soc.*, 1916, 38, 1216.

<sup>4</sup> *J. Amer. Chem. Soc.*, 1909, 31, 66; 1917, 39, 462, 470, 1018; 1918, 40, 813; 1919, 41, 1141.

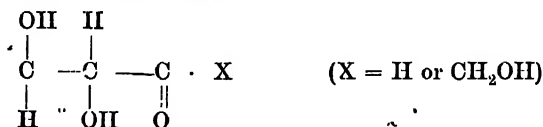
In the alkyl glucosides the same relation should hold; but now  $A$  is changed to  $A'$ , whilst  $B$  remains as before, and consequently  $A' + B$  and  $-A' + B$  will represent the two forms, and the sum or  $2B$  should be the same as the values for the corresponding hexoses.

	$[M]_D$	$\Sigma(2B)$		$[M]_D$	$\Sigma(2B)$
$\alpha$ -D-glucose	196	232	$\alpha$ -D-galactose	252	347
$\beta$ -"	36		$\beta$ -"	95	
$\alpha$ -Methyl D-glucoside	305	243	$\alpha$ -Methyl D-galactoside	379	379
$\beta$ -"	-62		$\beta$ -"	0	

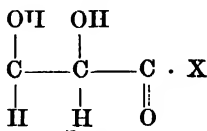
Similar results have been obtained by comparing the glucose pentacetates with the tetracetyl methyl glucosides, with the same satisfactory agreement.<sup>1</sup>

On this basis Hudson has been able to calculate the rotations of the  $\alpha$ -glucoside from its  $\beta$ -form and vice versa, and also the value of the attached group. From results of these observations he concludes that the  $\alpha$ -hexoses and hexosides may be distinguished from the  $\beta$ -isomers by the fact that, by deducting the  $\beta$  from the  $\alpha$  values, a positive quantity should be obtained.

Anderson<sup>2</sup> has extended these ideas and noted that the monosaccharoses containing the group



are strongly dextro-rotatory, that the reverse positions of the hydroxyls and hydrogen atoms produce strong laevo rotation, whereas the configuration in which both hydroxyls appear on the same side of the chain are weakly laevo- and dextro-rotatory



Hudson<sup>3</sup> has also pointed out that the rotations are high in the case of the sugars, lactones, and glucosides ( $[\alpha]_D = 20^\circ$  to  $150^\circ$ ), but low in that of the corresponding acids and alcohols ( $[\alpha]_D = 0^\circ$  to  $11^\circ$ ).

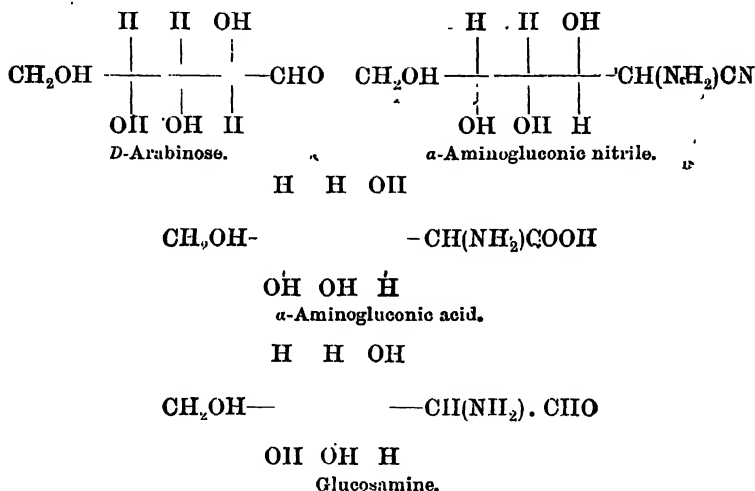
<sup>1</sup> Hudson and Dale, *J. Amer. Chem. Soc.*, 1915, 37, 1264.

<sup>2</sup> *J. Amer. Chem. Soc.*, 1911, 33, 405, 1510.

<sup>3</sup> *J. Amer. Chem. Soc.*, 1910, 32, 333, 345.

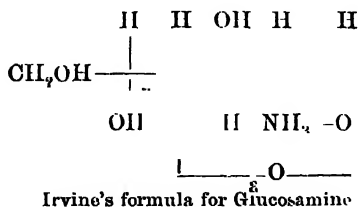


**Glucosamines.** *D*-Glucosamine is most conveniently obtained by the hydrolysis of chitin (the hard shell of crustaceae) with hydrochloric acid, and forms long colourless needles. Glucosamine hydrochloride exists in two forms,  $\alpha$  and  $\beta$ , and so also does the pentacetate, prepared by heating the hydrochloride with acetic anhydride and sodium acetate. It has been synthesised by Fischer and Leuchs<sup>1</sup> from *D*-arabinose, which is converted successively into the  $\alpha$ -aminogluconic nitrile,  $\alpha$ -aminogluconic acid, and, by reduction of the lactone of the latter, into *D*-glucosamine.



Glucosamine reacts abnormally with nitrous acid and gives a sugar named *chitose*, which is probably a furfural derivative.

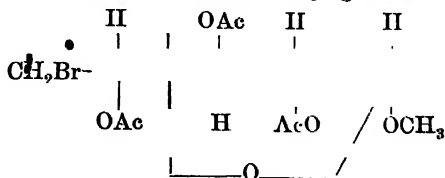
Irvine and Hynd<sup>2</sup> have eventually succeeded in converting glucosamine into glucose by a long series of operations, but the formula given to glucosamine is different from the above and is based on the betaine type of compound.



<sup>1</sup> *Fr.*, 1903, 36, 24.

<sup>2</sup> *Trans. Chem. Soc.*, 1912, 101, 1128; see also Levene, *J. Biol. Chem.*, 1916, 26, 367.

A second glucosamine<sup>1</sup> was obtained by Fischer and Zach<sup>1</sup> by the action of liquid ammonia on triacetylmethyl glucoside bromhydrin ;



Triacetyl methyl glucoside bromhydrin.

but its formula is not known with certainty. Fructosamine, a third isomer, has already been mentioned (p. 12).

**Structure of the Disaccharoses.** If the disaccharoses are structurally related to the alkyl glucosides, that is, if they are ether combinations of one hexose with another, they should exhibit similar properties—in other words, they should exist in two isomeric forms corresponding to  $\alpha$ - and  $\beta$ - and possibly  $\gamma$ -glucosides and capable of differentiation by enzyme action.

As each monosaccharose may be present in  $\alpha$ ,  $\beta$ , and  $\gamma$  forms, and each pair may be linked up in two ways, it is clear that a large number of modifications is possible.

Furthermore, it might be anticipated that their synthesis would be effected by the method used in the preparation of the glucosides. Each of these deductions has been in turn verified by Fischer and his collaborators. By the action of hydrochloric acid upon glucose in the cold, Fischer<sup>2</sup> obtained a disaccharose very similar to maltose, but unlike the latter, amorphous and non-fermentable by yeast. It was named *isomaltose*. E. F. Armstrong has since shown that some maltose is also present.

The relation of the disaccharoses to the  $\alpha$ - and  $\beta$ -glucosides has been established by E. F. Armstrong<sup>3</sup> by the same method which he applied to  $\alpha$ - and  $\beta$ -glucose, namely, by observing the rise or fall of rotation of the glucose isolated by the enzyme, when equilibrium was established. Using maltase and invertase as hydrolysing enzymes, it was shown that maltose and cane-sugar are  $\alpha$ -glucosides, lactose is a  $\beta$ -glucoside, raffinose is an  $\alpha$ -fructoside of melibiose, whilst starch, which is hydrolysed by diastase, appears to be a  $\beta$ -maltoside. The structural formulae originally assigned by Fischer to cane-sugar, lactose, maltose, and melibiose have since undergone some modification (see p. 57).

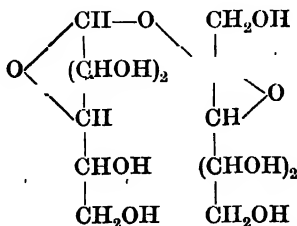
<sup>1</sup> Ber., 1911, 44, 132.

<sup>2</sup> Ber., 1890, 23, 3688.

<sup>3</sup> Trans. Chem. Soc., 1903, 85, 1305.

The formulae are based partly on the relation of the disaccharoses to the alkyl glucosides, partly on the general chemical behaviour of the sugars themselves. Thus, cane-sugar has no reducing properties and forms neither hydrazone nor osazone, whereas maltose, lactose, and melibiose behave in this respect like the aldo-hexoses. The difference between maltose, lactose, and melibiose is determined by the space configuration of the individual hexoses which are united in the molecule. The formula does not, however, indicate which half of the molecule in lactose and melibiose represents glucose and which galactose. Fischer and Armstrong<sup>1</sup> have found a simple way of solving the problem by forming the osone of the disaccharose and then hydrolysing it; the resulting hexosone will be that of the aldose constituent. Thus lactose and melibiose appear to be glucose-galactosides.

**Structure of Sucrose (Cane-Sugar).** Haworth, by introducing a new method of methylation (the use of methyl sulphate in presence of alkali) has succeeded in methylating the disaccharoses and thereby thrown new light on the structure of these substances. Thus, octamethyl sucrose, unlike cane-sugar, does not exhibit inversion on hydrolysis, for it yields a mixture of tetramethyl glucose of  $[\alpha]_D = +84^\circ$  and tetramethyl fructose of  $[\alpha]_D = +31^\circ$ , or a mean rotation of about  $[\alpha]_D = +57^\circ$ , whereas the tetramethyl fructose obtained directly from fructose has a rotation of about  $[\alpha]_D = -121^\circ$ , which if present in the mixture would produce inversion and give  $[\alpha]_D = -19^\circ$ . Moreover, the new tetramethyl fructose in its sensitiveness towards permanganate appears to belong to the  $\gamma$ -glucose or ethylene oxide type. Sucrose is therefore represented by the formula:<sup>2</sup>



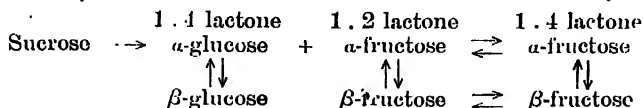
Structure of Sucrose.

Its conversion into glucose and fructose will therefore involve six

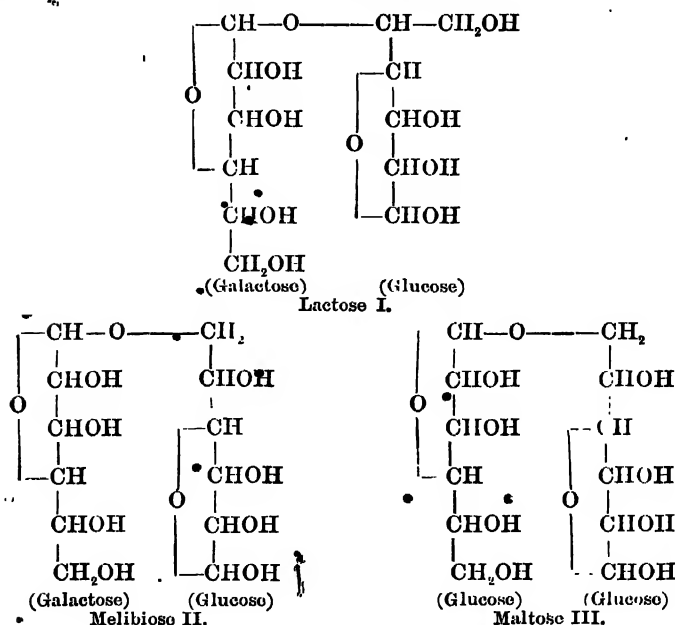
<sup>1</sup> Ber., 1888, 21, 2633; 1902, 35, 3141; see also Fischer and Meyer, Ber., 1889, 22, 362.

<sup>2</sup> Haworth and Law. Trans. Chem. Soc., 1916, 109, 1314; 1920, 117, 199.

changes, first a change of structure of fructose from the  $\alpha$  and  $\beta$  mixture of the 1.2 lactone to the equilibrium mixture of the  $\alpha$  and  $\beta$  1.4, lactone, and secondly a similar equilibrium change of the  $\alpha$ -glucose (sucrose being an  $\alpha$ -glucoside) to the equilibrium mixture of  $\alpha$ - and  $\beta$ -glucose.



**Structure of Lactose, Maltose, and Melibiose.** In the same way Haworth and Leitch<sup>1</sup> have prepared a heptamethyl lactoside which on hydrolysis yields tetramethyl galactose and a new trimethyl glucose. Unlike that obtained from glucose, it gives no osazone, and this, together with other facts, have led to formula I. Melibiose, which is also a glucose galactoside, is probably represented by II, whilst maltose, which forms a heptamethyl maltoside and breaks up on hydrolysis into the tetra- and tri-methyl glucose derived from glucose, retains the formula originally assigned to it by Fischer.<sup>2</sup>

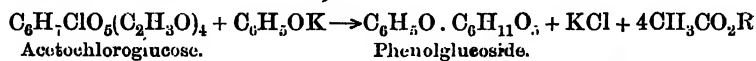


<sup>1</sup> Trans. Chem. Soc., 1918, 113, 188.

<sup>2</sup> Trans. Chem. Soc., 1919, 115, 809.



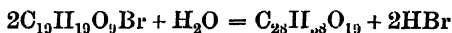
**Synthesis of the Disaccharoses.** The first synthetic disaccharose was obtained, as we have seen, by the action of hydrochloric acid on glucose, and although the method was used successfully in preparing a variety of phenol glucosides, when applied to the disaccharoses it proved to be unsatisfactory. Many years before, another process had been indicated by Michael,<sup>1</sup> who in 1881 obtained a phenol glucoside by the interaction of acetochloroglucose and potassium phenol in alcoholic solution.



The synthesis of cane-sugar described by Marchlewski<sup>2</sup> in 1899 is said to be based upon this method. Potassium fructosate and acetochloroglucose, dissolved in alcohol, were allowed to stand for a week, when cane-sugar, potassium chloride, and ethyl acetate were found to have been formed. But as the synthesis has never been confirmed on repetition of the process by others, it must be regarded for this and for other reasons as doubtful.

The new method devised by Fisher<sup>3</sup> in 1902 for the preparation of the acetochlorohexoses, by the action of liquid hydrogen chloride and bromide on the penta-acetates (p. 45), enabled him to apply Michael's reaction to effect the union of a variety of hexoses. By combining acetochloroglucose with sodium galactose, acetochlorogalactose with sodium glucose, and acetochlorogalactose with sodium galactose, three synthetic disaccharoses were created, all of which are hydrolysable by emulsin, whilst the second of the series, galactosido-glucose, is fermented by yeast and is probably identical with melibiose.

Another synthetic method introduced by Fischer and Delbrück<sup>4</sup> is to prepare the octacetyl disaccharose and hydrolyse the product. The octacetyl derivative was obtained in the case of glucose by shaking  $\beta$ -acetobromoglucose with silver carbonate in moist ether.



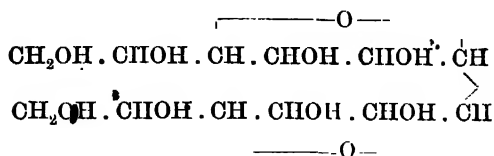
On hydrolysis with baryta water a disaccharose is formed which resembles trehalose but has a different rotation. It has been termed *isotrehalose*, and is probably one of the stereoisomers of the constitution:

<sup>1</sup> Ber., 1881, 14, 2097. <sup>4</sup>

<sup>2</sup> Trans. Acad. Sciences, Cracow, 1899.

<sup>3</sup> Ber., 1902, 35, 833, 3144, 3158.

<sup>4</sup> Ber., 1909, 42, 2776; 1910, 43, 2521.



Disaccharoses have also been obtained by the synthetic action of enzymes. Croft Hill prepared *isomaltosa*, a substance closely related to maltose by the action of maltase on glucose (p. 55), and Armstrong obtained maltose, using emulsin as enzyme. Fischer and Armstrong obtained an *isolactose* by the action of lactase on a mixture of glucose and galactose (p. 73), and Bourquelot and his co-workers' *gentiobiose* from glucose and *galactobiose* from galactose by the action of emulsin.

The following is a list of polysaccharoses :

Sugar.	Component monosaccharoses.	Rotatory power [α] <sub>D</sub>
<i>Disaccharoses.</i>		
Maltose	Glucose α-glucoside	+ 138°
Isomaltose	Glucose β-glucoside	—
Gentiobiose	Glucose β-glucoside	+ 9.6°
Cellobiose	Glucose β-glucoside	+ 34.6°
Lactose	Glucose β-galactoside	+ 52.5°
Isolactose	Glucose-galactoside	—
Melibiose	Glucose-galactoside	+ 143°
Turanose	Glucose + fructose	+ 71.8°
Sucrose	Glucose + fructose	+ 66.5°
Trehalose	Glucose + glucose	+ 167°
Vicianose	Glucose + arabinose	—
Glucosylose	Glucose + xylose	—
<i>Trisaccharoses.</i>		
Mannotriose	Glucose + galactose + galactose	+ 167°
Rhamninose	Glucose + rhamnose + rhamnose	— 41°
Raffinose	Galactose + glucose + fructose Sucrose β-galactoside	+ 104
Gentianose	Glucose + glucose + fructose	+ 33°
Melicitose	Glucose + glucose + fructose	+ 88.5°
<i>Tetrasaccharoses.</i>		
Stachyose	Fructose + glucose + galactose + galactose	+ 148°
Verbascose	Fructose + glucose + galactose + galactose	+ 169.9

**The Natural Glucosides.** This group of substances includes a variety of crystalline, and, for the most part, colourless compounds

<sup>1</sup> Bourquelot, Hérissey and Coirac, *Compt. rend.*, 1913, 157, 732; Bourquelot and Aubry, *Compt. rend.*, 1916, 165, 60.

of vegetable origin,\* which break up on boiling with mineral acids or by the action of specific enzymes into a sugar and into a second organic constituent. The sugar is, as a rule, *D*-glucose, but glucosides containing both active forms of arabinose, *L*-xylose, *D*-ribose, *L*-rhamnose, *D*-galactose, *D*-mannose, and *D*-fructose, as well as di- and tri-saccharoses, are known. Occasionally polyhydric alcohols may take the place of the sugar. The second constituent is usually an aromatic compound and includes a variety of phenols, aromatic alcohols, phenolic and hydroxy acids, coumarin derivatives and derivatives of anthraquinone, flavone, isothiocyanates, such as ordinary mustard oil, purine compounds, alkaloids, the indoxyl of indican (from indigo), the active principles of digitoxin and digitalin (from foxglove), and other substances of unknown constitution. The structure is probably similar to that of the simple alkyl glucosides, and, like these, they are divisible into  $\alpha$ - and  $\beta$ -series, as already explained (p. 39).

The functions of the glucosides in plants are at present somewhat obscure, but it is probable that they serve various purposes and that the enzyme associated with the glucoside hydrolyses it at the required moment and so stimulates some important change in plant metabolism.<sup>1</sup>

Among the substances which have in recent years been added to the list of glucosides is the tannin of galls. According to Fischer and Freudenburg<sup>2</sup> the purified material from Chinese galls contains about 7 to 8 per cent. of *D*-glucose, to which it owes its activity. As gluco-gallic acid has been isolated from the galls,<sup>3</sup> they were led to conclude that tannin is probably a pentadigalloyl glucose.



This view was confirmed later by the synthesis of pentadigalloyl glucose and other phenolic acyl glucosides which closely resemble tannin, but more especially by the synthesis of methyl tannin. Methyl tannin, obtained by the action of diazomethane on natural tannin,<sup>4</sup> is very similar if not identical with the product obtained by the action of pentamethyldigalloyl chloride on glucose, and probably consists of a mixture of the  $\alpha$ - and  $\beta$ -isomers.

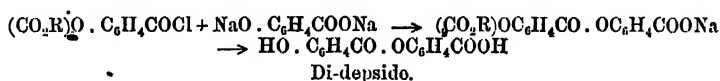
<sup>1</sup> H. E. and E. F. Armstrong, *Proc. Roy. Soc.*, 1910, 82 B, 588.

<sup>2</sup> *Ber.*, 1912, 45, 915, 2709; 1913, 46, 3253.

<sup>3</sup> Feist, *Abstr.*, 1913, i, 70.

<sup>4</sup> Herzig, *Ber.*, 1905, 38, 989.

The characteristic of the acid radical in these substances is the di-acid group which is formed by the union of the phenol hydroxyl of one phenolic acid with the carboxyl of a second. Fischer has named these substances *depsides* ( $\delta\epsilon\psi\epsilon\upsilon\varsigma$  = to tan) and distinguishes the number of grouped acid radicals by the prefix mono-, di-, tri-, &c. A general method for their preparation, devised by Fischer consists in protecting the phenol hydroxyl by introducing the carbethoxyl group (using chloroformic ester) and converting the acid into the chloride, the latter being then allowed to react with the sodium phenate of a second phenolic acid and so forth.\* The carbethoxyl group is finally removed by hydrolysis with dilute alkali.



The acid chloride of the depside is combined with glucose in presence of an organic base.

More recently Fischer and Bergmann<sup>1</sup> succeeded in preparing the *m*- and *p*-pentadigalloyl derivatives of both  $\alpha$ - and  $\beta$ -glucose by combining digalloyl chloride with glucose in presence of quinoline. They closely resemble the vegetable tannins in their tanning properties.

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<sup>1</sup> *Ber.*, 1918, 51, 1769, 1804; 1919, 52, 72.

## CHAPTER II

### FERMENTATION AND ENZYME ACTION

FERMENTATION may be broadly described as a process by which certain products are elaborated as the result of the activity of living cells. The lifeless products of the cells which directly induce these changes are termed *enzymes* (*εν ζύμη*, in yeast) and act either in the presence or absence of the living organism. Fermentation is therefore a result of which enzymes are the active cause.

Thus, the original meaning of the word, which was derived from *fervere*, to boil, and which connected it with the evolution of gas, has entirely disappeared.

**Historical.** The early history of fermentation is mainly concerned with alcoholic fermentation. Until the sixteenth century it was commonly supposed that the alcohol was present before fermentation in combination with impurities, which were removed during the process, thus liberating the alcohol. In 1682 Beecher proved that sugar was necessary for fermentation and that the alcohol did not pre-exist in the liquid. Towards the close of the eighteenth century Lavoisier demonstrated the true composition of cane-sugar, estimated its constituents, and followed quantitatively its conversion into alcohol and carbon dioxide. In 1837 an important advance was made by Cagniard de la Tour in France, and almost simultaneously by Schwann and by Kützing in Germany. They observed under the microscope the reproduction, by budding, of the small spherical bodies which had been seen many years previously by Leuwenhoek in the sediment or scum of fermenting liquids. They associated the disappearance of the sugar and the production of alcohol and carbon dioxide with the presence and reproduction of the organism. Notwithstanding the convincing nature of their experiments it was not until a quarter of a century later (1860) that the dependence of fermentation on the vegetative function of the yeast cell was generally accepted. The *vitalistic theory*, as it was called, had to contend against the strenuous opposition of Liebig and others of his school, who strove to explain the phenomena of life by the aid

of purely chemical and physical laws. Instead of crediting the yeast organism with the power of causing fermentation, they constructed a mechanical hypothesis, known as the *physical* or *vibration theory*. According to this hypothesis the real ferment was a non-living substance which rapidly decomposed, and in so doing transmitted a shock to the sugar molecules by which they were resolved into simpler substances. The theory was in fact a revival of the old views of Willis and of Stahl, which were put forward during the latter half of the seventeenth century. It was not long before a modification of Liebig's original conception was found necessary. The brilliant and conclusive researches of Pasteur left no doubt that the active agent in the change was the living cell. Efforts were made to bring the two views into harmony, and Naegeli put forward the theory that all substances capable of fermentation have their molecules in a state of active oscillation in virtue of their potential energy. The ferment, being in a similar condition, transmitted its motion to the fermenting material outside the living cell and thus effected its decomposition. The ferment itself was not supposed to be destroyed in the process, as Liebig had postulated in his original hypothesis.

At length, after nearly twenty years of fruitless discussion, Pasteur was able to definitely prove that alcoholic fermentation was essentially an intracellular change effected by living yeast, and to show that the lactic and butyric fermentations were kindred phenomena caused by individual organisms distinct from the yeast plant.

**Meaning of Fermentation.** Until comparatively recently a more or less sharp line of demarcation was drawn between a change, such as fermentation by yeast, and those reactions which are brought about by substances to which the name *enzyme* has been given. Emulsin, an enzyme found in bitter almonds, was observed as early as 1830 by Robiquet and Boutron, and its mode of action was elucidated in a striking way by Liebig and Wöhler.<sup>1</sup> They showed that the enzyme occurred along with the glucoside, *amygdalin*, and that when the cells of the almond are ruptured in the presence of water the two substances are brought into contact, with the result that the glucoside is resolved into benzaldehyde, hydrocyanic acid, and glucose.

By suitable methods it is possible to separate the enzyme from the glucoside and from the cells of the almond, and the reaction may consequently be carried out under conditions which preclude the possibility of any living material being concerned in the change.

<sup>1</sup> *Ann. Chim. Phys.*, 1837, 64, 185.

A similar reaction had been observed as far back as 1814 by Kirchoff, who discovered the conversion of starch into sugar by the presence of a watery extract of germinating barley, and twenty years later Payen and Persoz were able to roughly isolate the enzyme, to which they gave the name *diastase*. A similar enzyme was also discovered in the salivary secretion by Leuchs, and about the same time *pepsin*, an enzyme which brings about the hydrolysis of proteins, was found by Schwann in the gastric juice.<sup>1</sup>

Ferment reactions were therefore divided into two classes—reactions induced by *organised ferments* connected with the presence of living cells, and those caused by *unorganised ferments* capable of acting in a sterile medium.

This distinction persisted for a long time, although suggestions had been made that the difference between the two kinds of change was more apparent than real.<sup>2</sup> In 1896 E. Buchner was able to show that by rupturing yeast cells by mechanical means and expressing the cell juice under high pressure, a liquid is obtained which is capable of converting a not inconsiderable amount of sugar into alcohol and carbon dioxide in the complete absence of living yeast cells. This result at once placed alcoholic fermentation—the fermentation *par excellence*—upon a level with the changes produced by unorganised ferments. Further research has shown that by similar methods other organisms, particularly those which cause the lactic and acetic fermentations, may be made to yield crude enzyme preparations which are free from living cells, but are still capable of inducing the particular change associated with the organism from which they were derived.

The tendency is therefore to ascribe fermentation reactions, whether associated with living cells or not, to the structureless, non-living enzymes, although it must be admitted that many fermentations are known which cannot at present be shown to take place in the absence of living matter. It is therefore better to designate ferments as *intracellular* or *extracellular* according as they normally operate within or without the cell by which they are formed, not forgetting that in very many cases it is possible by artificial means to cause intracellular enzymes to exert their functions independently of the cell.

<sup>1</sup> The discovery of the ferments concerned in gastric digestion must really be ascribed to Réaumur (1752) and the Abbe Spallanzani (1785). The latter caused birds of prey to swallow small sponges attached to a string. After withdrawal, the sponges yielded a small quantity of gastric juice which was able to dissolve and change fragments of meat. These results were, however, not accepted as correct until many years later.

<sup>2</sup> Moritz Traube, *Pogg. Annalen*, 1858, 103, 331.

Enzymes are substances of the utmost importance to all living matter. They may be regarded as the 'chemical' reagents' of the organism. Their presence, even in minute quantities, effects the disintegration of large amounts of complicated substances of the most varied kind into simpler bodies, which are disposed of or utilized according to the requirements of the cell. They also furnish the means whereby the sun's energy, through the medium of plant life, is made available for the animal organism.

**Fermentation, a Catalytic Process.** The similarity existing between enzyme actions and ordinary catalytic or contact changes had not escaped the notice of Berzelius. The view of Ostwald that catalysis is essentially an increase in the velocity of a reaction, which normally proceeds at a definite though extremely small rate in the absence of a foreign substance or catalyst, is quite in harmony with all that is known of enzymes; so that an enzyme may be defined as a catalyst produced by living organisms. Moreover, like most catalytic processes, the speed of fermentation increases with the amount of enzyme, whilst the enzyme does not appear in the product nor does the quantity taking part in the reaction bear any definite molecular relation to the substance acted on. Another and striking analogy between inorganic catalysts and enzymes is the reversibility of certain ferment actions. The recent work which has been carried out upon both inorganic and organic catalysts, by the application of physical methods to the measurement of reaction velocities, has only served to emphasize their close connection. The mechanism of enzyme action is briefly discussed on p. 95.<sup>1</sup>

**Chemical Action of Enzymes.** The majority of enzyme reactions are of a simple hydrolytic character and the energy exchanges are small (e.g. the saponification of fat, the conversion of starch into sugars, the liberation of sugars from glucosides), and most of them can be carried out equally well with inorganic catalysts such as acid or alkali. Even some of the more complicated ferment changes, such as the oxidation of alcohol to acetic acid and the conversion of calcium formate into hydrogen, carbonic acid, and calcium carbonate, may be effected by finely divided metals such as platinum and iridium. It must, however, be remembered that, unlike most of the inorganic catalysts, the enzymes are to a considerable extent 'specific', e.g. a fat-hydrolysing enzyme is incapable of hydrolysing starch or glucosides. The two kinds of catalysts differ in another respect.

<sup>1</sup> Asher and Spiro, *Ergebnisse der Physiologie*, 1908, 1, 134; Bredig, *Anorganische Fermente*, *Habilitationsschrift*, Leipzig, 1904; Victor Henri, *Les Lois Générales de l'Action des Diastases*, Paris, A. Hermann, 1903; Senter, *Proc. Roy. Soc.*, 1905, 74, 201; Euler, *Zeit. physiol. Chem.*, 1905, 45, 420.



At a certain stage before complete hydrolysis is effected, the action of the enzyme often slows down. Diastase hydrolyses starch to maltose, when the process almost ceases before the glucose stage is reached; pepsin, in the same way, produces peptones when further hydrolysis to amino-acids proceeds so slowly that prolonged action may be necessary to detect it. Finally, there is an optimum concentration, above and below which there is a falling off in enzyme activity. There is a large amount of indirect evidence to support the view that an enzyme enters into a *definite combination* with the substance which undergoes change. It should be pointed out that if such a combination takes place with gain or loss of energy, it will change the energy content of the system and so alter the equilibrium-point attained.

E. F. and H. E. Armstrong<sup>1</sup> regard the enzyme as having a two-fold function, attracting the hydrolyte as well as effecting its hydrolysis, the double action being attributed to an 'acceptor', which attaches the hydrolyte and is similar if not identical with it and an acid catalyst (probably carboxyl of the protein group) which acts as the hydrolytic agent.

It is usually assumed, from analogy with other catalysts, that a minimum quantity of enzyme is capable of causing the decomposition of an infinitely large amount of the substance upon which it acts, provided that the necessary time is allowed. Practically, however, this condition of things is seldom approached, although in a few cases—notably the action of invertase upon cane-sugar and of the clotting enzyme, rennet—the enzyme is capable of bringing about change in as much as four hundred thousand times its own weight of substance without losing its activity. It is, however, much more usual for a considerable destruction of the enzyme to take place during the reaction, and it is a significant fact that the conditions most favourable to the rapid action of many enzymes are precisely those which promote their destruction. For example, the protein-dissolving enzyme, trypsin, secreted by the pancreas, acts most readily at a moderately high temperature and in a comparatively strong alkaline medium, and under these conditions the enzyme is rapidly destroyed, particularly in the absence of protein upon which it may act. In other cases the products formed during the reaction act prejudicially. Thus, the free acid, liberated from fats by animal lipase, has a distinctly harmful effect upon the enzyme. It follows that there is a definite limit to the amount of change that a certain quantity of enzyme can bring about; but the fact is not necessarily opposed to the action being catalytic.

**Composition of Enzymes.** It has not been possible to isolate an

<sup>1</sup> *Proc. Roy. Soc.*, 1913, 86B, 565.

enzyme in the pure state, and up to the present it has been impracticable to do more than investigate the effects produced when mixtures containing them are allowed to act upon substances of known composition. Of the enzymes themselves we know extremely little. In many cases it is possible to obtain solid amorphous preparations, which furnish extremely active enzyme solutions when dissolved in water. Unfortunately there is no definite criterion of purity to serve as a guide, and the methods available for the preparation of enzymes are such as would not remove many known impurities. The reason for this is that enzymes belong to the class of colloids—that is, they do not, as a rule, form true solutions, but are present as suspensions of fine particles which, when strongly illuminated by a side-light, reflect it and can be seen in the field of the microscope (ultra-microscope) as bright specks floating in the liquid.

The properties of colloids depend mainly on the large surface of the particles in proportion to their size. It has been shown that if a dissolved substance lowers the surface tension of a liquid there is a tendency for the substance to accumulate at the interspace between solid and solution. It can be readily understood that such a process must occur when a solution is in contact with colloidal substances whereby the latter tend to withdraw the substance from solution. The phenomenon is called 'adsorption', and it will occur whenever the surface energy of a solution, from whatever cause, is lowered. Colloids may possess an electric charge which may promote or diminish adsorption. For example, if arsenious sulphide, which forms a colloidal solution and carries an electronegative charge, and colloidal ferric hydroxide, which is electropositive, are mixed in such proportion that the mixture is electrically neutral, both are precipitated as a complex colloid. Very similar effects are produced by electrolytes. Colloids carrying a definite charge will be precipitated by ions of opposite sign.

Thus enzymes, being colloids, carry down by adsorption substances present in the solution from which they are precipitated.<sup>1</sup> It is not, therefore, surprising to find a portion of the solute firmly adhering to the enzyme in the process of purification by precipitation. The colloidal state of the enzyme no doubt produces in the same way a union with the substance acted on, which then undergoes chemical change (see p. 97). This view of the initial process of enzyme action seems to be generally accepted.

As a rule enzymes are thrown out of solution on addition of alcohol or salts, such as ammonium or sodium sulphates. Frequently they may be carried down with neutral precipitates, such as calcium phos-

<sup>1</sup> *Physics and Chemistry of Colloids*, by E. Hatschek. J. and A. Churchill, London, 1913.

phate, when formed in their presence. The precipitation of an alkaline solution of a protein, such as casein, with a weak acid, is often effective. In some cases, particularly that of the protein-hydrolysing enzymes, they may be withdrawn from solution by shaking with insoluble substances such as blood-fibrin, charcoal, cholesterol, or magnesium carbonate.

Generally speaking it is found that the most carefully purified enzyme preparations give the reactions of proteins, and for a long time the view was prevalent that enzymes belonged to the class of nucleo-proteins (p. 165). It is certain, however, that many enzymes are known which are not nucleo-proteins, and some, such as the vegetable oxidases, seem to contain scarcely any nitrogen.<sup>1</sup> In short, the view that enzymes are proteins is being gradually abandoned. The general properties of enzymes lead to the conclusion that they are unstable substances of very high molecular weight, and there are good reasons for believing that their molecules are asymmetric. In solution they are almost all destroyed at temperatures of about 65–80°, but in the absence of water they are more stable, and some have been heated to 150° without losing their activity.

It is probable that the enzymes will be found to be allied to the class of substances upon which they act. The enzymes which act upon the xanthine bases are intimately connected with nucleo-proteins, which themselves furnish xanthine bases upon hydrolysis. The purest pepsin yet obtained was found by Pekelharing<sup>2</sup> to have an elementary composition not differing widely from most proteins (C = 52, H = 7, N = 14.3, S = 1.65) and to resemble the nucleo-proteins in some other particulars. Trypsin, papain, and thrombase, all enzymes which act upon protein substances, would also appear to resemble complex protein derivatives, and this view is supported by the fact that the three proteases, trypsin, pepsin, and papain, are able to mutually 'digest' one another. According to O'Sullivan and Tompson,<sup>3</sup> invertase contains a carbohydrate, possibly in combination with a protein, though the purest preparations contain very little nitrogen.<sup>4</sup> Diastase,<sup>5</sup> however, appears to be a protein derivative.

Enzyme preparations invariably contain small quantities of inorganic matter, which in some cases seems to be of great importance. A striking example of this is seen in 'laccase', an oxidising enzyme

<sup>1</sup> The difficulties attending the satisfactory isolation of enzymes has led some investigators to doubt their real existence as definite substances. Arthus (*La Nature des Enzymes*, Thèse, Paris, 1896) expresses the view that enzymes represent no material substances, but are forms of imponderable energy comparable with light, electricity, &c. Others have tried to draw analogies between the enzymes and the emanations of radio-active substances, but these hypotheses are of course purely speculative, and there are at present no reasons why the older materialistic view should be abandoned.

<sup>2</sup> *Zeit. physiol. Chem.*, 1902, 35, 8.

<sup>3</sup> *Trans. Chem. Soc.*, 1890, 57, 834.

<sup>4</sup> Wróblewski, *Ber.*, 1908, 31, 1130.

<sup>5</sup> Euler and others, *Zeit. physiol. Chem.*, 1910, 69, 152.

discovered by Yoshida,<sup>1</sup> which plays an important part in the production of lacquer varnish from the sap of the lac tree. Bertrand<sup>2</sup> finds that the ash contains up to 2 per cent. of manganese, and that the activity of the enzyme is proportional to the manganese present. It was further shown that in certain laccase preparations, which contained an exceptionally low amount of manganese, the action of the enzyme could be increased thirtyfold by addition of manganese salts. It has been suggested that manganese, being able to form compounds of varying degrees of oxidation, acts directly as an oxygen carrier, though salts of other metals cannot replace it. The occurrence of manganese in the ash of tea-leaves is probably connected with the presence of a similar enzyme.

Calcium salts are found to be essential to the action of some of the clotting enzymes, such as rennin and thrombase, whilst chlorine would appear to be a necessary constituent of pepsin. Phosphorus is often found in the numerous enzymes associated with nucleoproteins, though it is not certain that the phosphorus is actually part of the enzyme molecule.

**Conditions determining Enzyme Action.** Enzymes can act only within a limited range of temperature. Little or no action is observable at the freezing-point, and a temperature of about 60° usually causes a fairly rapid destruction of the ferment, whilst at somewhat higher temperatures their destruction is almost instantly complete. In general, the enzymes of animal origin act best at about blood-temperature (37°), whilst a temperature of about 25° is often favourable for vegetable enzymes. In some cases enzymes from cold-blooded animals seem to act best at a temperature of about 15°.

With few exceptions the enzymes can act only in almost neutral solutions, and in general a faintly acid medium is preferable to one with an alkaline reaction. Trypsin, however, works well in a solution containing 1 to 2 per cent. of sodium carbonate, whilst, on the other hand, pepsin is most active in the presence of 0.2 per cent. of hydrochloric acid. Additions of most foreign substances affect enzyme reactions adversely, but occasionally small quantities of neutral salts are advantageous. Many neutral substances which are very poisonous for the living organism (cyanides, fluorides, chloroform), or aromatic hydrocarbons (toluene, &c.), are not nearly so prejudicial to the enzymes, so that substances of this kind are frequently added to solutions in which enzyme changes are in progress in order to prevent bacterial contamination.

<sup>1</sup> *Trans. Chem. Soc.*, 1883, 43, 472.

<sup>2</sup> *Compt. rend.*, 1897, 124, 1032.

**Specific Action of Enzymes.** The simplest reactions brought about by enzymes may be divided into three classes—those which bring about a simple hydrolysis, those concerned in oxidations and reductions (oxidases and reductases), and those which induce clotting of certain substances. The enzymes of the first class may be arranged according to the nature of the substance, or *substrate*, which undergoes change—polysaccharoses, di- and tri-saccharoses, glucosides, proteins and their decomposition products, purine bases, &c.

The nomenclature now in general use for the naming of enzymes is that suggested by Duclaux, and consists in adding the suffix *-ase* to the substance upon which the action of the enzyme was first observed. Certain of the older names, however, such as trypsin and emulsin, &c., are still retained.

*Enzymes which induce the hydrolysis of Polysaccharoses.*

Enzyme	Substrate.	Products.	Occurrence. <sup>1</sup>
Diastase (syn. Amylase)	Starch Glycogen	Dextrins Maltose	Veg. { Germinating grain and many plants, fungi, and bacteria Animal { Pancreatic juice, liver, saliva <sup>2</sup>
Inulase	Inulin	Fructose	Germinating bulbs and tubers. <i>Aspergillus niger</i>
Cellulase (syn. Cytase)	Cellulose	Reducing sugars	Veg. { Germinating grain, fungi Animal { Livers of carp and snails
Pectinase Gelase	Pectins Gelose (Agar-Agar)	Reducing sugars Reducing sugars	Germinating grain Bact. gelaticus
Caroubinase	Caroubin	Reducing sugars	C&rob beans

Amongst the enzymes concerned in the hydrolysis of the polysaccharoses, diastase is by far the most important. It has an extraordinarily wide distribution, the enzymes from both animal and vegetable sources being apparently identical. Relatively pure diastase is easily prepared by extracting green or air-dried malt with dilute spirit, and then precipitating the filtered liquid with absolute alcohol. The crude product is purified by reprecipitation and is freed from most of the inorganic impurities by dialysis. The substance finally obtained is a white powder, of which a very

<sup>1</sup> Only the principal sources of the enzymes can be noted here. A complete account of the distribution would occupy too much space.

<sup>2</sup> The name 'ptyalin' is commonly given to salivary diastase.

small quantity is able to liquefy a very large amount of starch paste. When allowed to act upon starch paste it is found that after a short time the liquid no longer gives a blue colour with iodine, but a deep red-brown, whilst at a later stage no reaction at all is observable. No definite conclusion as to the nature of the products of the action of diastase upon starch has yet been reached, but it is certain that maltose is the main product. A number of substances belonging to the class of dextrins (erythrodextrin, achroodextrin, amylo-dextrin, maltodextrin, &c.) have been described by different investigators, but they are difficult to characterize, and much confusion exists as to their individuality.<sup>1</sup> It was formerly believed that iso-maltose was also produced in the reaction, but this view is no longer generally accepted. Vegetable and animal diastase act equally well upon glycogen and upon starch, and the products are similar.

Inulase,<sup>2</sup> an enzyme occurring in the growing tubers of the artichoke and other plants, is able to bring about the conversion of inulin into fructose, but is without action upon starch. The other enzymes of this group, although of the utmost importance from a biological standpoint, possess little chemical interest, as the nature of the reactions involved is still obscure.

*Enzymes inducing the hydrolysis of the Di- and Tri-saccharoses.*

<i>Enzyme.</i>	<i>Substrate.*</i>	<i>Products.</i>	<i>Principal Occurrence.</i>
Maltase (syn. $\alpha$ -Glucase)	Maltose	Glucose	Yeast, malt, taka-diastase, intestinal juice
Invertase (syn. invertin, sucrase)	Cane-sugar	Fructose, glucose	Yeast extracts, many parts of plants, fungi, intestinal juice
Lactase	Lactose	Glucose, galactose	Kephir organism and some saccharomyces, intestinal juice
Trehalase	Trehalose	Glucose	Penicillium glaucum Aspergillus niger Green malt
Raffinase	Raffinose	Melibiose and fructose	Yeast and Aspergillus niger
Melibiose	Melibiose	Galactose, glucose	Froberg yeast
Melizitase	Melizitose	Touranose, glucose	Aspergillus niger
Touranase	Touranose	Glucose	Aspergillus niger

<sup>1</sup> Lintner and Düll, *Ber.* 1893, 26, 2533; Brown and Morris, *Trans. Chem. Soc.*, 1889, 55, 462; 1895, 69, 709.

<sup>2</sup> Green, *Annals of Botany*, 1, 223.

Maltase is one of the most important and widely distributed of the enzymes concerned in carbohydrate metabolism. It is found in most varieties of yeast and other fungi, and also in intestinal juice. Its special action is to convert maltose into two molecules of glucose; it is without action upon cane-sugar. Its occurrence along with diastase in malt extracts explains the frequent presence of glucose amongst the degradation products of starch.

Maltose is not directly fermentable by yeast; for it is necessary for the maltase present in the yeast to transform the maltose into glucose before conversion into alcohol and carbonic acid can occur. Since maltase is not readily obtained from yeast, unless the cells are thoroughly dried and then ground with sand and water, it follows that the conversion of maltose into glucose by yeast is an intracellular change.

Similarly it is found that, before maltose can be made use of as a source of energy by the animal organism, it must be first converted into glucose, a change which is brought about by the enzyme present in the intestinal juice.

The action of maltase upon maltose is of special interest, for it was thought by Croft Hill<sup>1</sup> to be reversible (p. 99). He found that glucose in concentrated solutions under the influence of maltase is partly converted into maltose. The same equilibrium mixture of maltose and glucose is produced whichever sugar is taken as the starting-point, and is dependent upon the concentration of the solutions. With solutions containing less than 4 per cent. of sugar no reversion is observable. Some discussion has taken place as to the exact nature of the sugars formed from glucose. Emmerling<sup>2</sup> considered that isomaltose is formed, whereas Croft Hill, on the other hand, maintained that maltose and a new disaccharose, *revertose*, are produced. It seems probable, according to E. F. Armstrong,<sup>3</sup> that revertose is identical with isomaltose, as both are hydrolysed by emulsin, and are therefore  $\beta$ -glucosides. It is at least certain that a synthetical process has been brought about by the enzyme.

Maltase is also able to bring about the hydrolysis of the  $\alpha$ -series of synthetical glucosides obtained by Fischer, to which reference will be made later (p. 74).

Trehalase is an enzyme which converts trehalose into glucose, and in most respects resembles maltase.

Invertase is a comparatively stable enzyme which, when acting under favourable conditions, can convert more than one hundred

<sup>1</sup> *Trans. Chem. Soc.*, 1898, 73, 634; 1903, 83, 378.

<sup>2</sup> *Ber.*, 1901, 34, 600.

thousand times its weight of cane-sugar into glucose and fructose. It has no action upon maltose and lactose, but apparently can attack gentianose, a complicated sugar which contains a cane-sugar grouping. Invertase appears to play an important part in connection with the photo-synthetic production of carbohydrates in green plants. According to Brown and Morris<sup>1</sup> cane-sugar is the first stable carbohydrate formed in the synthesis, and as cane-sugar, like maltose, does not seem to be directly assimilated by the organism, it is converted into the assimilable glucose by means of the invertase, which is commonly found in the green parts of plants.

Invertase is also found in the intestinal juice of most animals, and cane-sugar taken by the mouth is rapidly converted into glucose before absorption. The necessity for this is seen in the fact that cane-sugar, when injected intravenously, is excreted, unchanged, in the urine.

Lactase is the enzyme specially concerned in conversion of lactose into galactose and glucose. It is found in the intestinal juice, and occasionally in the pancreatic secretion of animals. It is much more abundant in young mammals than in adults, but the production of lactase may be induced by continued ingestion of lactose. Lactase is also found in extracts from the kephir organisms (milk-sugar yeast) and in certain saccharomyces. It is remarkable that it is commonly accompanied by invertase, but seldom if ever by maltase. Its function is similar to maltase and invertase, and, like the former, its action has been shown to be reversible. Fischer and Armstrong,<sup>2</sup> by acting upon a mixture of galactose and glucose with kephir lactase, obtained a disaccharose, isolactose, which is very closely allied to lactose. A disaccharose was also formed when glucose, but not galactose, was present.

The decomposition of raffinose (melitriose) into melibiose and fructose is accomplished by the enzyme *raffinase*. The melibiose may be further hydrolysed by an enzyme—*melibiase*—and yields glucose and galactose. Melibiase may possibly be identical with maltase. A similar hydrolysis of a trisaccharose is met with in the conversion of melizitose into glucose and the disaccharose, *touranose*. The latter sugar is converted into glucose by means of a separate enzyme.

<sup>1</sup> *Trans. Chem. Soc.*, 1893, 63, 604.

<sup>2</sup> *Ber.*, 1902, 35, 3114.



*Enzymes inducing hydrolysis of Glucosides.*

Enzyme.	Substrate.	Products.	Principal Occurrence.
Emulsin (syn. $\beta$ -glucase)	Amygdalase <sup>1</sup>	Glucose + <i>d</i> -mandelo nitrile	{ Bitter almonds Aspergillus niger Other plants
	Prunase	<i>d</i> -Mandelo nitrile	
	Lotase	Lotoflavin, glucose, and hydrocyanic acid	
	Myrosin	Sinigrin (Potassium Myronate)	Cruciferae and other orders
	Gaultheriase (syn. Betulase)	Gaultherin	Leaves of Gaultheria pro- cumbens and varieties of Azalea and Spiraea
Indigo ferment (Indimulsin)	Indican	Indoxyl and glucose	Indigo plants
Tannase	Tannins	Galic and ellagic acids, glucose	Aspergillus niger and galls
Rhamnase	Xantho- rhamnin	Rhamnetin, rhamnirose	Seeds of Persian berry
Erythrozyme	Ruberythric acid	Alizarin and glucose	Madder plant

**Enzymes causing hydrolysis of Glucosides.** Amongst the hydrolysing enzymes emulsin is by far the most important and the most widely distributed. It has the property of hydrolysing not only amygdalin, as already mentioned, but also a large number of other glucosides, the most important of which are given in the table on p. 41).<sup>2</sup>

Emulsin is also able to act upon certain synthetical glucosides. It will be remembered that by the action of various alcohols upon sugars in the presence of hydrochloric acid, Fischer was able to prepare two series of stereoisomeric glucosides, and that the  $\alpha$ -glucosides are exclusively attacked by maltase whereas the  $\beta$ -glucosides are exclusively attacked by emulsin (p. 40).<sup>3</sup>

It has been possible from these results to draw conclusions as to the configurations of some of the natural sugars and their derivatives. Maltose is clearly to be regarded as an  $\alpha$ -glucoside, for it is hydrolysed by maltase and not by emulsin, whilst the observation

<sup>1</sup> In many cases an enzyme may cause the hydrolysis of different substances, as in the case of emulsin, which can bring about the decomposition of a whole series of glucosides. In the tables, however, only one typical example can be given.

<sup>2</sup> A more complete list is given in *The Simple Carbohydrates and Glucosides*, by E. F. Armstrong, Monographs on Biochemistry, 2nd ed. Longmans, 1912.

<sup>3</sup> Fischer, *Zeit. physiol. Chem.*, 1898, 26, 61.

## HYDROLYSIS OF GLUCOSIDES

that emulsin from bitter almonds brings about the hydrolysis of lactose, and, further, that the enzyme lactase from kephir acts upon  $\beta$ -methyl glucosides, suggests that lactose is related to the  $\beta$ -glucosides.

Alkyl glucosides, derived from non-fermentable sugars such as the pentoses and heptoses, are unattacked by both enzymes (p. 41).

It appears at first sight a curious anomaly that amygdalin,

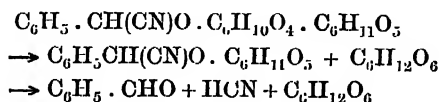
Glucoside.	Products of Hydrolysis.
Amygdalin	2 mol. glucose, 1 mol. hydrocyanic acid, 1 mol. benzaldehyde
Mandelo-nitrile glucoside (Prunasin)	1 mol. glucose, 1 mol. hydrocyanic acid, 1 mol. benzald. hyde
Coniferin	1 mol. glucose, 1 mol. coniferyl alcohol $\begin{array}{c} \text{OH} \\   \\ \text{CH}_3\text{O} \end{array} \text{C}_6\text{H}_5 \cdot \text{CH} : \text{CH} \cdot \text{CH}_2\text{OH}$
Salicin	1 mol. glucose, 1 mol. saligenin $\begin{array}{c} \text{OH} \\   \\ \text{C}_6\text{H}_4 \end{array} \text{CH}_2\text{OH}$
Arbutin	1 mol. glucose, 1 mol. quinol
Glucovanillin	1 mol. glucose, 1 mol. vanillin $\text{C}_6\text{H}_5(\text{OH}) \cdot (\text{OCH}_3) \cdot \text{C} \begin{array}{l} \text{O} \\ \diagup \\ \text{H} \end{array}$
Helicin	1 mol. glucose, 1 mol. salicylaldehyde
Glucovanillic acid	1 mol. glucose, 1 mol. vanillic acid $\begin{array}{c} \text{HO} \\   \\ \text{CH}_3\text{O} \end{array} \text{C}_6\text{H}_3 \cdot \text{CO}_2\text{H}$
Daphnin	1 mol. glucose, 1 mol. daphnetin $3.4.(\text{OH})_2 \cdot \text{C}_6\text{H}_2 \begin{array}{c} \text{CH} : \text{CH} \\   \\ \text{O} \end{array} \text{CO}$
Aesculin	1 mol. glucose, 1 mol. aesculetin $4.5.(\text{OH})_2 \cdot \text{C}_6\text{H}_2 \begin{array}{c} \text{CH} : \text{CH} \\   \\ \text{O} \end{array} \text{CO}$
Phloridzin	1 mol. glucose, 1 mol. phloretin (the phloroglucinol ester of <i>p</i> -hydroxyhydratropic acid)

which from Fischer's early investigations\* was considered to be a maltoside, and under the influence of maltase was converted into mandelo-nitrile glucoside, should at the same time be attacked by emulsin like a  $\beta$ -glucoside.

It now appears that maltase of yeast and emulsin contain a common enzyme, *amygdalase*, which does not attack  $\beta$ -glucosides. The other enzyme of emulsin, which is occasionally found alone, as in the leaves of the cherry laurel, is called *prunase*, and hydrolyses mandelo-nitrile glucoside.

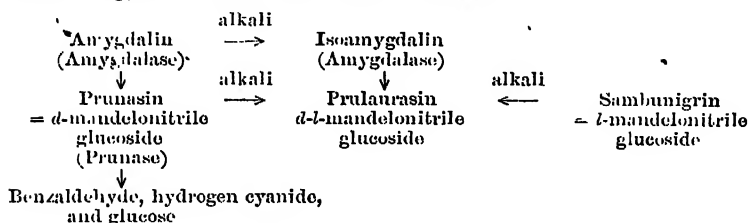
Moreover, the disaccharose of amygdalin is not maltose but a sugar

probably related to trehalose. It thus comes about that amygdalase first breaks up the amygdalin into *d*-mandelonitrile glucoside, which is then attacked by prunase, forming benzaldehyde, hydrogen cyanide, and glucose.



The latter glucoside has recently been found in certain plants and is named prunasin. Both amygdalin and prunasin are racemised on heating with caustic soda, the former yielding isoamygdalin and the latter the natural glucoside, prulaurasin or *d*-*l*-mandelonitrile glucoside, which is also formed by the action of amygdalase on isoamygdalin. The laevo enantiomorph of prunasin is also found in nature, as sambunigrin, in the leaves of the common elder.<sup>1</sup>

The following table, slightly modified from that given by E. F. Armstrong,<sup>2</sup> will make these relations clear:



It is also probable that the hydrolysis of the fructosides and galactosides is brought about by specific enzymes, occurring together with the enzymes which act upon the glucose derivatives.

Until recently emulsin was the only enzyme known which produced hydrocyanic acid as a result of its action, but recently, the 'cyanogenetic' enzymes have been discovered, notably *lotase* by Dunstan and Henry,<sup>3</sup> and *gynocardinase* by Power and Lees.

The other enzymes of the class under consideration are not of such general interest as emulsin, although the glucosides upon which they act are of the most diverse kinds, as will be seen from the table. The enzyme which acts upon the glucoside 'indican' is of considerable industrial importance. It was formerly believed that indigo-white was formed in the reaction, but Hazewinkel<sup>4</sup> states that the product is indoxyl. Other enzymes which apparently belong to this class have been described by Schweitzer. They

<sup>1</sup> Both these substances have been synthesized in active and racemic forms by E. Fischer, *Ber.*, 1917, 50, 1047.

<sup>2</sup> *The Simple Carbohydrates*, by E. F. Armstrong. Longmans, Green, 1912.

<sup>3</sup> Dunstan and Henry, *Proc. Roy. Soc.*, 1901, 67, 224; 68, 374.

<sup>4</sup> *Chem. Ztg.*, 1900, 24, 409.

bring about the hydrolysis of substances known as *kolanin* and *cacaonin*, the products being either caffeine or theobromine together with glucose and a substance known as kola-red.

*Enzymes causing the hydrolysis of Proteins, their derivatives,  
and the Purine bases.<sup>1</sup>*

Enzyme.	Substrate.	Products.	Principal Occurrence.
Trypsin	Proteins, proteoses and peptones, polypeptides	Amino-acids and simpler polypeptides	Pancreatic secretion
[Enterokinase Pepsin -	Trypsinogen Proteins and some albuminoids	Trypsin { Proteoses Peptones Amino-acids	[Intestinal juice] Gastric secretion
Papain	Proteins	Amino-acids	Juice of Carica Papaya
Bromelin	Proteins	Amino-acids	Pine apple fruit
Erepsin	Proteoses, peptones	Amino-acids	Intestinal mucosa
Arginase	Arginine	Ornithine and urea	Liver and kidney
Guanase	Guanine	Xanthine	Spleen, thymus, and liver
Adenase	Adenine	Hypoxanthine	Spleen, thymus, and liver

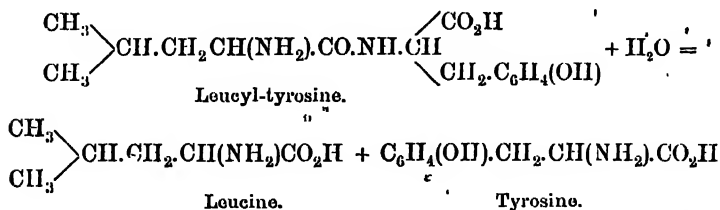
Trypsin and pepsin are the two most important enzymes connected with protein hydrolysis. Their action is in many respects similar, and their points of difference are quantitative rather than qualitative.

Pepsin is the enzyme chiefly concerned in gastric digestion. It is secreted by glands in the mucous membrane of the stomach, and acts best in the presence of hydrochloric acid (0.2 per cent.), which is also secreted by special cells in the mucous membrane. Pepsin, under ordinary conditions, causes a rapid hydrolysis of the complex proteins with the formation of proteoses and peptones. If, however, the digestion is very prolonged the latter are to some extent decomposed and the simple amino-acids, such as leucine, tyrosine, &c., are formed.

Trypsin, on the other hand, acts best in an alkaline medium, and, although proteoses and peptones are formed as intermediate products, they rapidly undergo further hydrolysis producing amino-acids, together with certain *polypeptides* containing three or four amino-acid radicals linked together (see p. 151). Trypsin is also able to cause the hydrolysis of many of the synthetical polypeptides referred to on p. 154, particularly those obtained from the

<sup>1</sup> To understand the nature of the proteins and purine bases, the student should read the two chapters which follow.

amino-acids of higher molecular weight. Thus, leucyl-tyrosine yields tyrosine and leucine, and the reaction is probably typical of most of the changes involved in the hydrolysis of a complicated protein.



The action of trypsin, it should be added, is selective; for Fischer and Abderhalden<sup>1</sup> have shown that whereas alanyl glycine, for example, is hydrolysed, glycyl-alanine is not, and of these hydrolysable polypeptides only certain of the active forms are attacked by the enzyme, as will be seen from the following table:

<i>Hydrolysed.</i>	<i>Not Hydrolysed.</i>
<i>d</i> -Alanyl <i>d</i> -amino	<i>d</i> -Alanyl <i>l</i> -alanine
<i>d</i> -Alanyl <i>l</i> -leucine	<i>l</i> -Alanyl <i>d</i> -alanine
<i>l</i> -Leucyl <i>l</i> -leucine	<i>d</i> -Leucyl <i>l</i> -leucine
<i>l</i> -Leucyl <i>d</i> -glutamic acid	<i>l</i> -Leucyl <i>l</i> -leucine

It is noteworthy that, like the natural sugars, which are fermented by yeast, it is only the polypeptides composed of the active amino-acids occurring in nature that are acted upon by trypsin.

More definite evidence is available as to the successive stages in the elaboration of trypsin than is the case with any other enzyme. The production of granules, which are believed ultimately to furnish trypsin, may be observed in the cells of the pancreas. The pure secretion of the pancreas is, however, practically devoid of action upon proteins; but if it be mixed with a trace of intestinal juice a digestive fluid of extraordinary power is obtained. The researches of Pawlow and of Bayliss and Starling<sup>2</sup> have conclusively shown that the intestinal mucosa furnishes an enzyme *enterokinase* which has the property of converting the inactive trypsinogen, the precursor or 'zymogen' of trypsin, present in the pancreatic juice, into the active trypsin. Enterokinase is therefore a 'ferment of ferments', and at present no other similar enzyme is known.

The secretion of pancreatic juice, and therefore of trypsin, is not

<sup>1</sup> *Zeit. physiol. Chem.*, 1905, 46, 53; 1907, 51, 264.

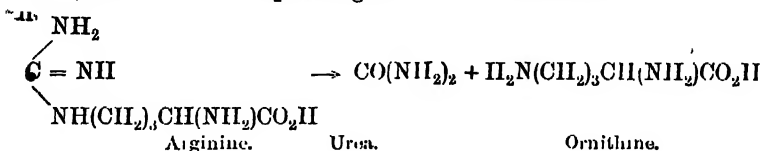
<sup>2</sup> Bayliss and Starling, *Journ. Physiol.*, 1904, 30, 61; 1905, 32, 129.

continuous, but is regulated by a simple chemical mechanism according to the needs of the organism. Bayliss and Starling<sup>1</sup> have been able to prove that the acid in the food passing from the stomach liberates a definite chemical substance, *secretin*, from the cells of the duodenal mucous membrane, and this, upon absorption, evokes a profuse secretion of the pancreas, containing all the enzymes normally present.

Besides trypsin and pepsin many other enzymes are known which cause the hydrolysis of proteins. Most animal tissues contain intracellular proteases, which are also found in many plants and fruits (papain, bromelin) and in some bacteria. The 'carnivorous plants' are also able to hydrolyse their protein food by means of enzymes.

A peculiar enzyme has been described by Cohnheim,<sup>2</sup> which is said to have the property of causing the hydrolysis of proteoses and of peptones, but is without action upon the ordinary proteins. It occurs in the small intestine, and its action is supposed to supplement that of trypsin, but it has not been very carefully studied.

Arginine is constantly present in the products of hydrolysis of proteins. An enzyme *arginase*, which occurs principally in the liver, is able to decompose arginine into ornithine and urea:



Arginase is also able to convert guanidine into urea and ammonia. It is the only urea-forming enzyme which has so far been isolated, but it is very probable that others exist.<sup>3</sup>

Many tissues also contain enzymes which are able to remove ammonia from the amino acids resulting from protein hydrolysis. This reaction is of great biological importance, but it has not yet been possible to follow the changes completely.

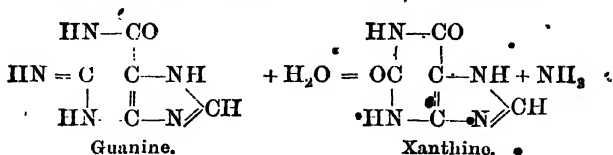
The question of the existence of enzymes which act upon the purine bases (see p. 116) has recently attracted much attention, and at least one definite enzyme, *guanase*, has been isolated by Jones,<sup>4</sup> which is able to convert guanine into xanthine with loss of ammonia.

<sup>1</sup> *Journ. Physiol.*, 1902, 28, 325.

<sup>2</sup> *Zeit. physiol. Chem.*, 1902, 35, 134.

<sup>3</sup> Kossel and Dakin, *Zeit. physiol. Chem.*, 1904, 41, 321.

<sup>4</sup> *Zeit. physiol. Chem.*, 1904, 42, 35 and 343.

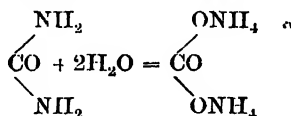


In a similar fashion adenine is converted into hypoxanthine, although it is not quite certain whether this change is due to the same enzyme that acts upon guanine or to a specific *adenase*.<sup>1</sup>

There is also some evidence of the existence of enzymes occurring among the products of hydrolysis of nucleic acids which act upon the pyrimidine bases, such as cytosine.

The purine and pyrimidine bases are also attacked by oxidising enzymes accompanying guanase and adenase, and will be referred to under the head of 'oxidases'.

Two important enzymes, which have not yet been considered, are urease and lipase. Urease converts urea into ammonium carbonate.



The enzyme is found in media in which the *micrococcus urae* or certain other organisms have grown, and is responsible for the ammoniacal fermentation of stale urine. The enzyme readily diffuses from the cells of the dead organisms, and may be roughly isolated by precipitation of the filtered liquid with alcohol. The conversion of ammonium carbonate into urea—the opposite change to that brought about by urease—is known to take place in the animal body. The change may be shown to occur when a 'surviving liver' is perfused with blood containing ammonium carbonate or carbamate, but, up to the present, it has not been possible to isolate the enzyme or to demonstrate the reaction except in the presence of intact liver cells.

Lipase is a widely distributed enzyme, and has the property of causing the hydrolysis of fats and certain derivatives of fats such as lecithin. It is found in the pancreatic juice, liver, and blood of animals, and in most oily seeds, particularly during germination. There appear to be distinct differences between the enzymes from different sources. Lipase is not only able to hydrolyse fats, but also many esters, such as ethyl acetate, ethyl carbonate, ethyl salicylate, salol, &c. If a *dl*-ester of an asymmetrical acid be hydrolysed by lipase, the two forms are attacked at unequal rates;<sup>2</sup>

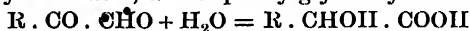
<sup>1</sup> Cp. Schittenhelm, *Zeit. physiol. Chem.*, 1905, 45, 121 and 152.

<sup>2</sup> Dakin, *Journ. of Physiol.*, 1903, 30, 253; 1905, 32, 199; *Proc. Chem. Soc.*, 1903, 19, 161.

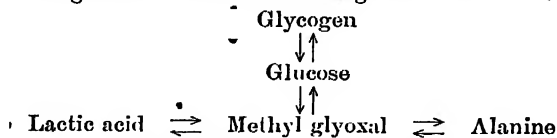
a similar selective hydrolysis has been observed by Fischer in the hydrolysis of racemic polypeptides by trypsin. The action of lipase has been shown to be reversible (see p. 99).

It has been stated that lipase may be separated into two substances—a heat-stable dialysable substance and an unstable non-dialysable substance. Neither body alone is able to bring about the hydrolysis of esters, but the reaction takes place in the presence of both substances. Many other facts are known which support the idea that enzyme activity in general is due to two or more substances acting conjointly, and in some cases the second substance or *co-ferment* appears to be inorganic (see p. 86).

A very important hydrolytic enzyme has been recently found by Dakin and Dudley<sup>1</sup> in various tissues and secretions of the animal body and in smaller quantity in yeast and a few vegetable tissues. It has the property of converting the glyoxals into the corresponding optically active lactic acids, and is named *glyoxalase*. Methyl glyoxal gives ordinary lactic acid, whilst phenylglyoxal yields mandelic acid.



It is an interesting fact that the pancreas not only contains none of the enzyme, but inhibits its action. What the significance of this fact may be is not yet explained, but the presence of lactic acid as a normal constituent of muscle suggests methyl glyoxal as its precursor, which in turn may be derived from a carbohydrate, or may represent the first step in the metabolism of amino acids. The following series of reversible changes has been demonstrated:



*The Oxidising Enzymes (Oxidases).*

Enzyme.	Substrate.	Products.	Principal Source.
Alcohol oxidase	Alcohol	{ Acetic acid Acetaldehyde Acids	Bact. Xylinum
Aldehyde	Aldehydes		Bact. Aceti, &c.
Tyrosinase	Tyrosine	{ Homogentisic acid, Black colouring matter	Plants, e.g. Dahlia, certain fungi
Laccase	Urushic acid	Varnish	Juice of lac-tree
Xanthine-oxidase	{ Xanthine Hypoxanthine	Uric acid	Liver and spleen
Uricolase	Uric acid	Allantoin <sup>2</sup>	Liver
$\beta$ -Oxybutyrase	Hydroxybutyric acid	Acetoacetic acid	Liver

<sup>1</sup> Journ. Biol. Chem., 1913, 14, 555; 1913, 15, 155, 423, 463.

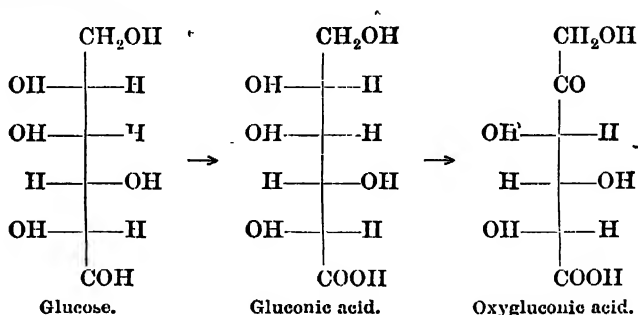
<sup>2</sup> Beitr. chem. phys. u. path., 1907, 9, 295.



**The Oxidases.** The oxidases are an ill-defined class of enzymes, which enable free oxygen to effect oxidations which would not otherwise take place. Although oxidation must obviously play an extremely important part in the metabolism of living cells; our knowledge of the mechanism of the process is extremely meagre. This is partly due to the fact that it is only in comparatively few cases that the action of an oxidising enzyme has been followed under fairly normal conditions. It is well known that many animal tissues are able to convert, to some extent, aldehydes into acids, a change which has been studied with benzaldehyde, salicylaldehyde, and formaldehyde; the presence of oxidases is also inferred from the fact that the same tissues are able to form indophenol colouring matters when they are digested with an alkaline mixture of  $\alpha$ -naphthol and alkyl *p*-phenylenediamines. It is, however, questionable as to how far results obtained by reactions of this nature are comparable with those effected by the enzymes working under normal conditions and acting upon their usual substrates.

Acetic fermentation is one of the simplest biochemical oxidations, and is readily brought about by *bacillus aceti* and other bacteria. The conversion of alcohol into acetic acid by the organism appears to take place in stages, as acetaldehyde is frequently formed. The bacillus aceti is not only able to oxidise alcohol, but can convert glucose into gluconic acid, glycol into glycollic acid, and mannitol into fructose. Another organism, *B. xylinum*, which commonly causes acetic fermentation is said to be identical with the 'sorbose bacterium' of Bertrand, referred to on p. 38.

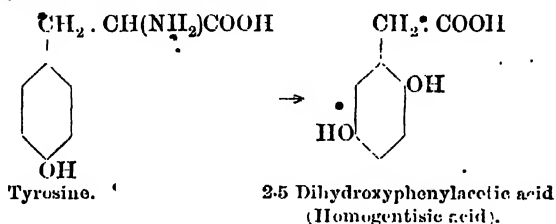
The sorbose bacterium, in addition to its action on polyhydric alcohols, is able to oxidise many aldehydes to the corresponding acids, and if, as in the case of gluconic acid, a secondary alcohol group, capable of attack, is present, the final product of the reaction will be a ketonic acid.



Oxidation in the absence of the living cells has been recently

observed by Buchner and Meisenheimer,<sup>1</sup> who were able to prepare an active preparation from the cells of the dead bacteria, and there can be little doubt that the active agents in these changes are enzymes, comparable in every way with those that cause alcoholic fermentation.

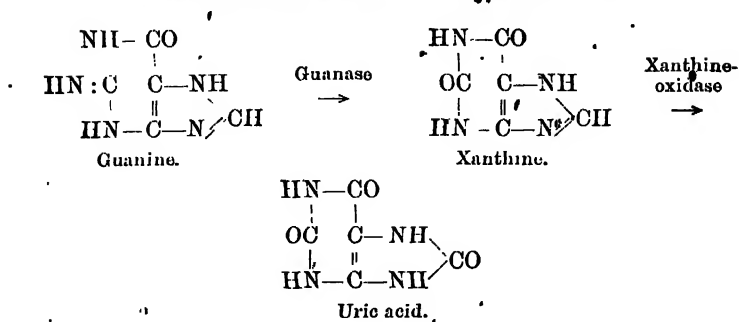
Tyrosinase and laccase are vegetable oxidases which are closely allied and often occur together. The former converts tyrosine ( $\alpha$ -amino- $\beta$ -hydroxyphenyl-propionic acid), a common constituent of proteins, into a black, insoluble substance, of unknown constitution, the formation of which appears to be preceded by that of 2-5 dihydroxyphenylacetic acid (homogentisic acid).



This reaction indicates a curious shifting of the position of the para hydroxyl group. It is of interest to note that in certain pathological conditions the tyrosine present in protein food is converted into homogentisic acid, which is found in the urine. Laccase converts urushic acid, contained in the light coloured juice of the lac tree, into a deep black varnish-like substance of unknown composition. Both tyrosinase and laccase are also able to oxidise polyhydric phenols, such as pyrogallol and quinol, but laccase is without action upon tyrosine.

Two distinct oxidases are known which are concerned in the oxidation of substances belonging to the purine group. The first is named *xanthine-oxidase*, and is able to bring about the oxidation of xanthine or hypoxanthine to uric acid, whilst the second enzyme carries the oxidation of uric acid further, forming products among which allantoin has been identified. Xanthine-oxidase occurs alone in the spleen, but both enzymes are found together in the liver. The existence of enzymes (guanase, adenase), which can convert guanine and adenine into hypoxanthine and xanthine has already been mentioned (p. 79), and it is therefore clear that by the combined action of these ferments it is possible to convert any of the four xanthine bases, commonly occurring in the animal body, into uric acid, a fact which is doubtless of great physiological significance.

<sup>1</sup> Ber., 1903, 36, 634.



The large part that glucose plays in cell metabolism long ago suggested that enzymes must exist which have the power of oxidising, or at least decomposing, the simpler sugars. A number of these bodies have been described under the name of *glycolytic enzymes*. Unfortunately, the experimental difficulties connected with this kind of investigation (arising from bacterial contamination) are so great that grave doubts are entertained as to the correctness of many of the results. Certain investigators, such as Stoklassa and Czerny,<sup>1</sup> assert that these enzymes are widely distributed; Cohnheim<sup>2</sup> and others are of opinion that their action may be observed in preparations obtained from a certain combination of tissue extracts, whilst Claus and Embden<sup>3</sup> have completely failed to confirm any of these results. Until, therefore, further experimental evidence is available, it is impossible to come to a definite conclusion, but it is at least certain that excellent *a priori* grounds exist for believing that important enzymes of this type actually occur.

It has usually been assumed that the colourless products resulting from the hydrolysis of indican were converted into indigo-blue, through the action of a specific oxidase contained in the leaves of the indigo plant; but the separate existence of this enzyme has recently been questioned.

That most vegetable and animal tissues possess the power of causing the decomposition of hydrogen peroxide has been known for a long time. It seems likely that this reaction is due to a special enzyme *catalase*, which has been roughly isolated in two different forms by Loew. Certain other oxidases, however, are also able to produce the same decomposition. Catalase has usually been classed amongst the oxidases, but it is questionable if the enzyme plays any part in oxidative processes; more probably, its function is to

<sup>1</sup> *Centralt. f. Physiol.*, 18, 793.

<sup>2</sup> *Zeit. physiol. Chem.*, 1904, 42, 401.

<sup>3</sup> *Beitr. z. physiol. u. path. Chem.*, 1905, 6, 214.

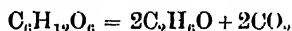
remove the traces of hydrogen peroxide, which are constantly formed as a by-product in other oxidations.

Many other vegetable oxidases have been described, including the so-called 'peroxidases' which activate hydrogen peroxide and possibly other peroxides, and so induce reactions which the peroxide alone could not accomplish.<sup>1</sup> Numerous systems for classifying these enzymes have been proposed, but they have at present a limited chemical interest, for it is not clear what type of reaction they normally induce nor upon what kind of substances they act.<sup>2</sup>

**Reductases.** The property which some animal tissues possess of effecting certain reductions, such as the conversion of the colouring matter, methylene blue, into a colourless leuco-base, has long been known; but it has not been possible to satisfactorily isolate the reducing agent. Rey-Pailhade has shown that certain organisms, such as yeast, are able to convert free sulphur into hydrogen sulphide, and this is believed by some to be caused by a reducing enzyme. The conversion of nitrates into nitrites and of nitrobenzene into aniline has also been carried out with extracts of animal tissues, but the satisfactory isolation of a reducing enzyme has not yet been accomplished.

**Alcoholic Fermentation.** Alcoholic fermentation has been studied more extensively than perhaps any other biochemical change, and a brief outline of some few of the earlier investigations has already been given.

The nature of the decomposition of glucose on fermentation, which was originally represented by the simple equation—



has in process of time resolved itself into a reaction of steadily increasing complexity. For, in addition to the higher alcohols included in the term fusel oil, Pasteur found glycerol and succinic acid to be the regular accompaniments of the process. Since then acetic, formic, and other acids, together with various aldehydes and esters, have been added to the list of by-products.

In the last few years new light has penetrated the haze in which the problem has so long been involved by the use of yeast juice in fermentation in place of the living cell. Under these conditions neither fusel oil nor succinic acid is found. This was followed by Ehrlich's discovery<sup>3</sup> of the origin of some of the higher alcohols of

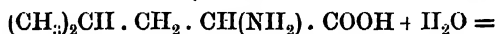
<sup>1</sup> Willstätter and Stoll, *Annalen*, 1918, 416, 21.

<sup>2</sup> A review of work upon the vegetable oxidases will be found in a 'Sammlung' by Bach and Chodat, *Biochem. Centralbl.* i, 1903, Nos. 11 and 12.

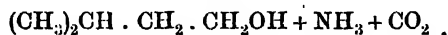
<sup>3</sup> *Ber.*, 1907, 40, 1047.

fusel oil. He has shown that certain of the amino-acids, which appear during the decomposition of the proteins present in beer wort and grape juice, undergo hydrolysis in presence of the living cells and yield alcohols with loss of carbon dioxide and ammonia, the latter being assimilated and removed.

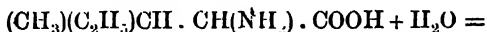
Leucine and isoleucine, both of which represent fragments of the protein molecule (p. 135), give respectively isoamyl and secondary butyl carbinol, which form the bulk of the fusel oil fraction—



Leucine.



Isoamyl alcohol.



Isoleucine.



Secondary butyl carbinol.

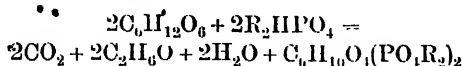
Succinic acid has been traced in the same way to glutamic acid, although the action here is not quite the same. For if the first part of the reaction determines the formation of the alcohol from the amino-acid, as shown above, an  $\alpha$ -hydroxy-acid and not a dibasic acid would be formed, involving thereby a further process of oxidation to complete the change. It has been pointed out that, as yeast contains an oxidase as well as a reductase, no difficulty need be felt in explaining the second stage of the process.

The higher alcohols being thus disposed of, we have still to account for the glycerol which makes its appearance, when yeast juice is used, to the extent of 3 to 4 per cent. of the sugar, as well as to explain the mechanism of the principal change effected during the fermentation of the glucose molecule, namely, the conversion of the latter into ethyl alcohol and carbon dioxide.

Since the discovery of yeast juice, which contains an enzyme or enzymes capable of producing fermentation, the latter process has been brought within the domain of enzyme actions. But although the fermentative process has, in a sense, been simplified by this discovery, it has at the same time been complicated by the observations of Harden and Young,<sup>1</sup> who showed that yeast juice may by dialysis be separated into two substances, an enzyme and a co-enzyme, which must act conjointly to bring about fermentation. Harden has further shown that the initial stages in the decomposition of the sugar mole-

<sup>1</sup> *Proc. Physiol. Soc.*, Nov. 1904, 1-2; *Journ. Physiol.*, 1904, 32, 1; *Proc. Chem. Soc.*, 1905, 21, 189.

cule are determined by the formation of a hexose phosphate under the agency of an enzyme hexose phosphatase, in which two molecules of hexose take part according to the equations—



Harden's interpretation of this equation is that in the presence of phosphate and of the complicated machinery of enzyme and co-enzyme, two molecules of the hexose, or possibly of the enolic form, are each decomposed primarily into two groups. Of the four groups thus produced, two go to form alcohol and carbon dioxide, and the other two are synthesised to a new chain of six carbon atoms, which forms the carbohydrate residue of the hexosephosphate. The introduction of the phosphoric acid groups may possibly occur before the rupture of the original molecules and may even be the determining factor of this rupture; or, again, this introduction may take place during or after the formation of the new carbon chain.<sup>1</sup>

If we examine the formulae of the four natural hexoses which undergo fermentation, using the aldehyde and ketone structure given on p. 36, it is clear that one part of the molecule is reduced at the expense of the other, the latter being thereby oxidised, or, in other words, that there is a transference of hydrogen in one direction and of oxygen in another to form alcohol and carbon dioxide.

Moreover, seeing that with yeast juice the reaction may proceed "out of contact with air, and that the great majority of enzyme reactions are hydrolytic, the natural conclusion—a conclusion adopted by Baeyer<sup>2</sup> and others—is that this transference of hydrogen and oxygen takes place either by the addition and removal of the elements of water, or else by some kind of isomeric change, or, finally, by a combination of the two.

Duclaux was the first to observe the formation of alcohol and carbon dioxide from sugar by the action of alkalis in presence of sunlight. The experiment was repeated by Buchner and Meisenheimer,<sup>3</sup> who obtained as much as 2 to 3 per cent. of alcohol. With more dilute alkali, however, they found that a different reaction occurred, and as much as 50 per cent. of the glucose was converted into inactive lactic acid together with a small quantity of formic acid, whilst about 40 per cent. consisted of a complex mixture of hydroxy-acids containing 4 to 6 carbon atoms. Moreover, Buchner and

<sup>1</sup> *Alcoholic Fermentation*, by A. Harden. Longmans.

<sup>2</sup> *Ber.*, 1870, 3, 63.

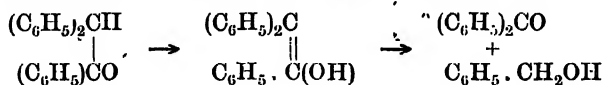
<sup>3</sup> *Ber.*, 1904, 37, 417. •

Meisenheimer found occasionally lactic acid among the products of fermentation with yeast juice, though never with living yeast.

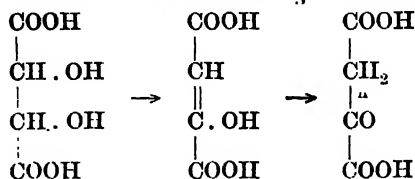
Duclaux, who afterwards obtained alcohol and calcium carbonate from calcium lactate in sunlight, was led to regard lactic acid as the direct, and alcohol and carbon dioxide as indirect, products of disintegration; and the same view was at one time adopted by Buchner and Meisenheimer until they and Sator proved by very careful experiments that lactic acid was incapable of fermentation either by living yeast or yeast juice.

Let us inquire further into the formation of lactic acid. As we have seen (p. 13), Lobry de Bruyn found that dilute alkalis, alkaline earths, and a few other metallic oxides convert any one of the three sugars, *d*-glucose, *d*-mannose, and *d*-fructose, into a mixture of the three, and the change has been ascribed to the intermediate formation of an enolic form which, by the addition and subsequent removal of a molecule of water, gives one or other of the above three substances (p. 44). Here, then, we have an isomeric change from keto to enol form in two directions (one in glucose and mannose and another in fructose) followed by the addition and subsequent removal of a molecule of water. Glyceric aldehyde and dihydroxy-acetone undergo, as Meisenheimer found, a similar isomeric change under similar conditions.

That such a reaction is not uncommon is shown by the fact, recently discovered by Marshall, that phenyl desoxy-benzoin breaks up spontaneously into benzophenone and benzyl alcohol.<sup>1</sup>



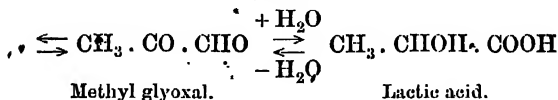
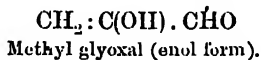
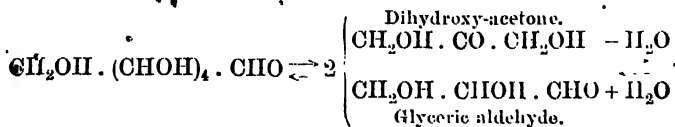
The reverse process has been observed by Wohl in the case of tartaric acid, which can be converted into oxalacetic acid whereby isomeric change follows instead of precedes the removal of water.



There seems little doubt, therefore, that such a process is a reversible one.

<sup>1</sup> *Trans. Chem. Soc.*, 1914, 105, 527.

Applying it to the conversion of glucose into lactic acid, as suggested by Wohl,<sup>1</sup> we shall have the following series of changes, all of which are probably reversible:



What evidence is there of the depolymerisation of the sugar molecule into two molecules of glyceric aldehyde (or dihydroxy-acetone) and of the intermediate formation of methyl glyoxal? The reply to the first is that glyceric aldehyde and dihydroxy-acetone may be condensed to form a hexose, and it may be confidently assumed that under appropriate conditions reversal occurs;<sup>1</sup> to the second, that Levene and Meyer<sup>2</sup> have proved that leucocytes convert *D*-glucose, *D*-mannose, and *D*-fructose into *D*-lactic acid, and can also transform methyl glyoxal into a mixture of inactive and *D*-lactic acid. Further, Wohl found that in alkaline solution methyl glyoxal readily passes into lactic acid. The reverse change has been shown to occur by Mandel and Lusk,<sup>3</sup> who found that lactic acid, when administered to diabetic animals, is converted into glucose, and by Dakin and Dudley,<sup>4</sup> who found that both methyl glyoxal and *D*-lactic acid are similarly transformed into glucose. The relation between glyceric aldehyde and dihydroxy-acetone on the one hand and methyl glyoxal and lactic acid on the other, has been demonstrated by Embden,<sup>5</sup> who showed that the two former undergo conversion into lactic acid in the liver, and by Meisenheimer, who found that the same two substances are transformed into methyl glyoxal on heating with dilute sulphuric acid.

<sup>1</sup> The supposed isolation of the methylphenyllosazone of dihydroxy-acetone recently announced by Boysen Jensen has been adversely criticized by Buchner and Meisenheimer. A. Fernbach, on the other hand, has observed the disintegration of glucose by living schizomycetes (*Tyroschizomycetes*) and its press juice into dihydroxy-acetone.

<sup>2</sup> *J. Biol. Chem.*, 1913, 14, 149.

<sup>4</sup> *J. Biol. Chem.*, 1913, 14, 555.

<sup>3</sup> *Amer. J. Physiol.*, 1906, 13, 129.

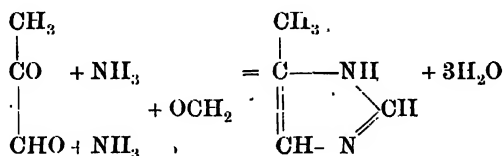
<sup>5</sup> *Biochem. Zeitsch.*, 1912, 25, 1.



The formation of methyl glyoxal from glucose has been proved in various ways.

Nef has given experimental evidence of the formation of methyl glyoxal by the action of caustic soda on glucose.<sup>1</sup>

Knoop and Windaus<sup>2</sup> found that when zinc ammonium hydroxide acts upon glucose in sunlight at the ordinary temperature a large quantity of methyl iminazole is produced, and the production of this substance is readily explained on the assumption of the intermediate formation of methyl glyoxal and of formaldehyde.



In presence of acetaldehyde dimethyliminazole is formed. The formation of methyl glyoxal from glucose has also been shown by Pinks,<sup>3</sup> who obtained the diphenylhydrazone by acting upon glucose in presence of phenylhydrazine, and by Dakin and Dudley, by distilling a glucose solution with sodium phosphate, when methyl glyoxal was found in the distillate. Moreover, Dakin and Dudley observed that when a solution of lactic acid is digested at 37° in presence of nitrophenylhydrazine, the nitrophenylosazone of methyl glyoxal is precipitated, and that, on the other hand, methyl glyoxal yields lactic acid by the aid of glyoxalase (p. 81).

Incidentally it may be observed that, as alanine gives methyl glyoxal by loss of ammonia, it might be expected to yield glucose, and both Lusk and Ringer, as well as Dudley and Dakin, have shown that glycosuric animals convert many amino acids into glucose. The contrary process—that is, the formation of ketonic aldehydes—may therefore represent the first step in the metabolism of amino acids.

Thus the chain of reversible reactions between glucose and lactic acid is nearly complete. The only missing links are the direct proof of the presence of glyceric aldehyde or dihydroxy-acetone and the reversibility of the glyceric aldehyde and methyl glyoxal change.

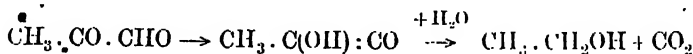
Assuming, for the sake of argument, that all the above changes have been completely demonstrated, we have to determine which products form the precursors of alcohol in fermentation. The forma-

<sup>1</sup> *Annalen*, 1904, 335, 254, 279.

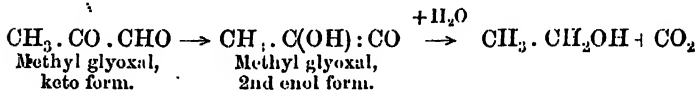
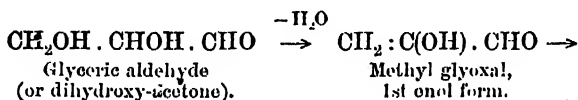
<sup>2</sup> *Ber.*, 1905, 38, 1166; 1906, 39, 3886; 1907, 40, 799.

<sup>3</sup> *Ber.*, 1898, 31, 31.

tion of alcohol and carbon dioxide from methyl glyoxal has been explained by Dakin and Dudley by supposing isomeric change to take place between the aldehyde and ketone group, and to be followed by the addition of the elements of water.



Thus, methyl glyoxal furnishes an important link between glyceric aldehyde or dihydroxy-acetone (which are mutually convertible, like glucose and fructose) on the one hand and alcohol on the other, the only assumption being that methyl glyoxal is capable of isomeric change in two directions after the manner of the hexoses.

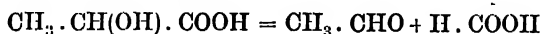


An important question still remains to be answered. If glyceric aldehyde or dihydroxy-acetone and methyl glyoxal represent intermediate stages in the process of fermentation, they should be attacked alike by yeast or yeast juice. Buchner and Moisenheimer<sup>1</sup> have tried the action of yeast and yeast juice upon the three substances in question and found that whilst dihydroxy-acetone is rapidly fermented (though much less rapidly than glucose) glyceric aldehyde is fermented slowly, whereas methyl glyoxal is not fermented at all.<sup>2</sup> This observation appears to negative the view that methyl glyoxal is the real precursor of alcohol.

<sup>1</sup> Ber., 1910, 43, 1773.

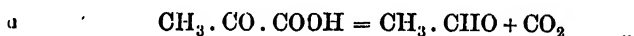
<sup>2</sup> Buchner and Moisenheimer were led from these experiments to regard dihydroxy-acetone and not glyceric aldehyde or methyl glyoxal as the precursor of fermentation, and explain the inactivity of lactic acid obtained from the former by reason of its symmetrical structure. An equally satisfactory explanation might be found in its formation from methyl glyoxal, which contains no asymmetric carbon atom. Lebedew's suggestion that dihydroxy-acetone first undergoes synthesis to a hexosephosphate is met by the fact that such a sugar would be an inactive mixture, and that only one active form could be fermented, whereas 90 per cent. is transformed.

There is, however, another view of fermentation, first put forward by Schade,<sup>1</sup> in which lactic acid is made the precursor of alcohol, 'breaking up, as it is known to do, with dilute sulphuric acid into acetaldehyde and formic acid.

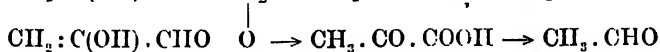
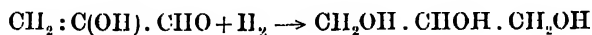


By the action of metallic rhodium on these two products, formic acid is converted into hydrogen and carbon dioxide, and Schade found that this liberated hydrogen reduces the aldehyde to ethyl alcohol. He suggests, therefore, that in fermentation acetaldehyde and formic acid may appear not necessarily from lactic acid, but from some labile substance yielding these two products.

Now, Neuberg and his co-workers have found that yeast contains an enzyme called *carboxylase*, which splits off carbon dioxide from  $\alpha$ -ketonic acids. Thus, pyruvic acid breaks up into acetaldehyde and carbon dioxide.



By introducing sodium or calcium sulphite into the fermenting solution, the aldehyde can be isolated as the bisulphite compound to the extent of 75 per cent. of the theoretical amount. At the same time the glycerol content is increased in the ratio of one molecular proportion. The explanation is that half the methyl glyoxal which is formed from the sugar is reduced to glycerol, whilst the other half is oxidised to pyruvic acid which yields acetaldehyde.<sup>2</sup>



**Acid Fermentations.** In addition to acetic fermentation, which has already been mentioned, several fermentations are known in which substances belonging to the carbohydrate group are converted into hydroxy-acids or acids of the acetic series. The production of acids, especially lactic acid, in many fermentative processes was early recognized, but it was reserved for Pasteur to show the connection

<sup>1</sup> *Zeit. physik. Chem.*, 1906, 57, 1.

<sup>2</sup> Neuberg and Reinfurth, *Ber.*, 1919, 52, 1677

existing between specific organisms and the production of lactic acid, and also to prove that the process was quite distinct from alcoholic fermentation. The power to produce lactic acid from sugars is not confined to the *B. acidi lactici* of Pasteur, but is shared by quite a large number of organisms of various kinds, though the relative yields of lactic acid vary enormously with different bacteria. The reaction is brought about by an enzyme, which has been shown to be active, even after the cells of the organism have been killed by acetone.<sup>1</sup>

Lactic acid may be formed from glucose, fructose, galactose, and at least one pentose, rhamnose; also from mannitol, dulcitol, and sorbitol, as well as from inositol (hexahydroxy-hexahydrobenzene). The manner in which lactic acid is produced from sugar, already discussed in connection with alcoholic fermentation, is not easy to understand. If the view there put forward is correct, the lactic acid should be inactive, as the asymmetry originally present in the sugar molecule is lost. Until recently this was commonly assumed to be the case, but McKenzio<sup>2</sup> has found that most fermentation lactic acid is distinctly dextro-rotatory. This result might, however, be explained on the basis of an initial formation of inactive lactic acid, followed by a selective conversion of a portion of the laevo-acid into other products. Frankland and MacGregor<sup>3</sup> have, in fact, shown that such a resolution of inactive lactic acid may be brought about by some organisms.

Butyric fermentation is associated with separate organisms from those that cause lactic fermentation, but the two classes of bacteria are frequently found together. Butyric acid is formed from lactic acid and also from a large number of those substances which readily yield lactic acid under the influence of bacteria. The mechanism of butyric fermentation, which is usually accompanied by the evolution of hydrogen, is much more complicated than that of acetic or lactic fermentation.

Under the influence of certain other organisms lactic acid may be made to yield other saturated acids, such as acetic, propionic, and valeric acids. Special organisms are also known which form citric and oxalic acids, when grown in glucose solutions, but the chemistry of these reactions is still obscure.<sup>4</sup>

<sup>1</sup> Buchner and Meisenheimer, *Ber.*, 1903, 36, 634; Herzog, *Zeit. physiol. Chem.*, 1903, 37, 381.

<sup>2</sup> *Trans. Chem. Soc.*, 1905, 87, 1373.

<sup>3</sup> *Trans. Chem. Soc.*, 1893, 63, 1028.

<sup>4</sup> A very large amount of work has been devoted to following the biochemical changes which the lower organisms are able to effect. Reactions like those

**Clotting Ferments.** Reference must be made to a curious class of enzymes which bring about the clotting of certain substances. The best known of these are *thrombase*, the blood clotting ferment, *rennin*, which causes the clotting of milk, and *pectase*, which acts upon certain complicated carbohydrates occurring in plants. In the case of all of these enzymes it is found that calcium salts play an important part in the reaction. In the case of thrombase the calcium salts appear to convert the zymogen, or precursor of the enzyme, into the active enzyme, just as enterokinase converts trypsinogen into trypsin, with the difference that enterokinase has itself the properties of an unstable enzyme. The calcium salts may be said to act as a 'co-ferment'. The clotting of blood is a phenomenon which has been closely studied by physiologists, and consists in the conversion of a colourless, globulin-like substance, *fibrinogen*, into a white stringy protein *fibrin*. It has been possible to roughly isolate the zymogen of the enzyme, and to observe its conversion into active thrombase on addition of calcium salts.

The action of rennin appears to be somewhat different. Rennin is chiefly derived from the gastric mucosa of mammals, and has been used for a very long time for curdling milk in the manufacture of cheese. The enzyme is formed from a zymogen by the action of the acids of the stomach, and not by the action of calcium salts, as in the case of thrombase. The clotting of milk or of a caseinogen solution involves two distinct reactions—firstly, the conversion by the enzyme of the caseinogen into a soluble protein closely allied to casein, and secondly, the conversion of this substance into an insoluble clot by the action of calcium salts.

If a solution of caseinogen, free from calcium salts, is digested with pure rennin no clotting occurs, but if traces of calcium salts are added, the liquid may be caused to clot even after the enzyme has been destroyed by boiling.<sup>1</sup> It is not certain whether the reactions brought about by the clotting enzymes involve simply a change in physical condition, or whether some chemical reaction takes place as well.

brought about by the nitrifying bacteria are of the utmost economic importance, whilst the substances produced by the different putrefactive and pathogenic bacteria have an important bearing upon the causation of disease. Reference should be made to works on Bacteriology for particulars of these and similar topics, which would be beyond the scope of this essay.

<sup>1</sup> To avoid confusion it is important to remember that many continental writers employ Hammarstein's nomenclature, and term the original substance acted upon by the enzyme 'casein' and the product of the action of the enzyme 'pa.acasein'.

**Anti-Enzymes.** Mention must be made of this interesting class of substances, the existence of which has only recently been definitely established. The anti-enzymes are of importance since they form a connecting link between the toxins and enzymes. It is well known that if an animal is injected with small but gradually increasing quantities of the poisonous toxins, extracted from the cells of an organism, such as that which causes tetanus, the animal rapidly acquires a degree of immunity, which in some cases is very great. It is found that this immunity is due to the production of definite substances, which may be found in the blood, and which have the power of combining with the toxin, and so preventing its further action. These bodies are known as anti-toxins, and are of the greatest importance in pathology. In a similar way it is found that injection of certain enzymes into an animal is followed by the appearance in the blood of substances which are able to inhibit the reaction normally brought about by the enzyme. For example, it is found that blood-serum containing anti-pepsin has a powerful action in hindering or almost entirely preventing the action of pepsin upon proteins. Some of these *anti-bodies* are of great importance; for their presence in the blood explains the resistance of protein-containing tissues of the body to the protein digesting enzymes of the alimentary tract.

• So far it has been possible to obtain anti-bodies for the following enzymes: trypsin, pepsin, lipase, emulsin, urease, lactase, tyrosinase, chrombase, and rennin. These anti-bodies are essentially specific—that is, they only inhibit the action of the particular enzyme used in their preparation. The ‘lock and key’ simile of Fischer might be applied to the enzymes and anti-bodies as well as to the enzymes and their substrates. It has been possible to distinguish enzymes such as animal and vegetable rennin which are otherwise indistinguishable, owing to the fact that their respective anti-bodies were not interchangeable, and this biological method of investigation will no doubt be of service in the future.

**Mechanism of Enzyme Action.**<sup>2</sup> It has already been pointed out that, according to the law of mass action applied to unimolecular, non-reversible changes, the following equation holds (Part I, p. 277):

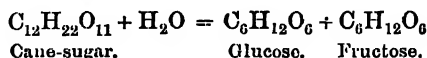
$$k = \frac{1}{t} \log \frac{a}{a-x}.$$

<sup>1</sup> Morgenroth, *Centralb. d. Bakter.*, 26 319; 27, 721.

<sup>2</sup> For the kinetics of enzyme action see *Chemical Statics and Dynamics*, p. 353, by J. W. Mellor. (Longmans.)

Wilhelmy,<sup>1</sup> in his classical investigation which appeared in 1850, showed that the inversion of cane-sugar by acids follows the mass law.

The process is represented by the following equation, which is apparently bimolecular:



But if the solution is dilute the amount of water is practically constant, and the reaction may therefore be treated as monomolecular and it is also non-reversible.

By observing the change of rotation on the addition of a minute quantity of hydrochloric acid the following numbers were obtained:

<i>t</i> in minutes.	Deviation.	Concentration per cent.	$\log a - \log (a-x)$ .	<i>k</i> .
0	+46.75°	65.45		
15	43.75	62.45	.0204	.00136
30	41.00	59.70	.0399	.00133
45	38.25	56.95	.0605	.00134
60	35.75	54.45	.0799	.00133
75	33.25	51.95	.1003	.00134
90	30.75	49.45	.1217	.00135
105	28.25	46.95	.1441	.00137
120	26.00	44.70	.1655	.00137
∞	-18.70			

The velocity (*k*) is constant, or, in other words, with a decreasing quantity of cane-sugar there is a proportionate decrease in the quantity inverted in unit of time, and the curve represented by the values of *t* and *x* is logarithmic.

Other acids give similar results, although the rate of inversion varies with different acids. As the rate of inversion appears to be directly proportional to the electrical conductivity of the acids in question, the process has been ascribed to the action of hydrogen ions, though it does not seem probable that the velocity of enzyme action is conditioned by the presence of ions.

The hydrolysis of cane-sugar by invertase was first submitted to quantitative examination by O'Sullivan and Thompson<sup>2</sup> in 1890. Using the optical method of Wilhelmy, they found that the velocity (*k*) increases slightly until 80 per cent. of the sugar had been hydrolysed and then decreases. Nevertheless, they drew the general conclusion that, in spite of these small deviations, the reaction follows the mass law. The subject was reinvestigated in 1902 by

<sup>1</sup> Pogg. Ann., 1850, 81, 413, 499; Ostwald's *Klassiker*, No. 29.

<sup>2</sup> Trans. Chem. Soc., 1890, 57, 834.

A. J. Brown,<sup>1</sup> who found that the velocity is not a constant, but that a given quantity of invertase decomposes a nearly constant weight of sugar in unit of time. Thus, in one hour, using different strengths of sugar solution, the following quantities were inverted :

Grams per 100 c.	Grams inverted.
4.89	1.230
9.85	1.355
19.91	1.355
29.96	1.235
40.02	1.076

This is contrary to the mass law, according to which the greater the concentration the greater should be the quantity inverted. It was further observed that, after a certain proportion of sugar had been inverted, the linear period (of equal weights in equal times) is succeeded by a logarithmic period (or a decreasing quantity in equal times). This change of velocity accompanying the inversion of cane-sugar in the earlier and later stages of the process was also observed by A. J. Brown<sup>2</sup> during the fermentation of sugar by yeast; by H. T. Brown and Glendinning<sup>3</sup> in the action of diastase on starch, and by E. F. Armstrong<sup>4</sup> during the hydrolysis of milk-sugar by the enzymes, lactase and emulsin, and of maltose by maltase. In each case there was a linear followed by a logarithmic period. During the logarithmic period the velocity is not necessarily constant, but, as in the case of milk-sugar, there is a fall, and in that of cane-sugar, when hydrolysed by invertase,<sup>5</sup> a rise in velocity-constant, as seen in the following table :

Hydrolysis of Cane-sugar.		Hydrolysis of Milk-sugar.	
Minutes.	Velocity-constant.	Hours.	Velocity constant.
66	0.00058	1	0.0610
168	64	2	513
332	72	3	460
488	77	5	310
696	85	24	129

This difference in velocity has been explained in certain cases as due to the liberation of some substance which either activates the enzyme (autocatalysis) or produces the opposite effect (negative autocatalysis). In the hydrolysis of proteins by trypsin, for example, the amino acids set free reduce the activity of the enzyme, which is more intense in the presence of a small quantity of hydroxyl ions. What is the reason of the slowing down of the process in the second period? It cannot be that the mass law is untrue, and the

<sup>1</sup> *Trans. Chem. Soc.*, 1902, 81, 373.

<sup>2</sup> *Trans. Chem. Soc.*, 1892, 81, 369.

<sup>3</sup> *Trans. Chem. Soc.*, 1902, 81, 388.

<sup>4</sup> *Proc. Roy. Soc.*, 1901, 73, 500.

<sup>5</sup> *The Nature of Enzyme Action*, p. 74, by W. M. Bayliss.



cause must be looked for elsewhere. A. J. Brown suggested that the substance undergoing change unites with the enzyme previous to decomposition, after which the enzyme is liberated, and can unite with more substance, and so forth. That such a union is probable has been shown by O'Sullivan and Tompson, who found that invertase survives a much higher temperature when cane-sugar is present than when absent; by Bayliss, who found that trypsin is much more stable in presence of its substrate; and by the observations of Fischer and others on the selective action of enzymes. This process may be initiated by adsorption, as suggested by Bayliss (see p. 66). If the amount of enzyme is large and the process of combination instantaneous, or very rapid in comparison with the subsequent rate of hydrolysis, the velocity ( $k$ ) would still be constant. The only change in the effect would be that the substance and enzyme would be changing together instead of the substance alone. If, however, the amount of enzyme is small compared with that of the substance, only a portion of the substance, namely that in union with the enzyme, would change, and, until the substance had diminished below a certain amount, equal quantities would undergo hydrolysis in equal times. Both A. J. Brown and E. F. Armstrong have shown that the curve becomes logarithmic or linear according to the proportion of enzyme present. Another possible cause affecting the mass law, also suggested by A. J. Brown, might be due to the accumulation of the products of hydrolysis which by union with the enzyme would withdraw it from its sphere of action. This view has received corroboration from the fact that invertase and trypsin are much more stable in presence of the products of their action, and the recent researches of E. F. Armstrong,<sup>1</sup> who has shown that the action of the enzyme is only retarded by the presence of those hexoses which are derived from the hydrolysis of the disaccharose or glucoside undergoing change.

Galactose, for instance, retards the hydrolysis of  $\beta$ -galactosides (e.g. milk-sugar) by lactase, whilst glucose and fructose are inert. The previous experiments of Henri<sup>2</sup> also appear to show that the retarding action of invertase on cane-sugar is determined by the nature of the product, for of the two, glucose and fructose, the latter alone retards the process. On the other hand, fructose retards the action of maltase on maltose more than glucose, and the latter does not apparently affect the action of emulsin on  $\beta$ -glucosides, whilst

<sup>1</sup> *Proc. Roy. Soc.*, 1904, 73, 516.

<sup>2</sup> *Compt. rend.*, 1901, 133, 891.

galactose retards the action of maltase on  $\alpha$ -glucosides, though not as much as glucose.

Finally, the process of reversion must be considered as a cause of retardation in enzyme action. The suggestion was first made by Croft Hill<sup>1</sup> when studying the hydrolysis of maltose by maltase. He found that not only the addition of glucose retards the process, but that maltase has the power of converting glucose into the isomeric disaccharoses, namely revertose and probably maltose (p. 72).

The various factors affecting the rate of enzyme action have been tabulated by Bayliss as follows:

Retardation	Acceleration.
Reversibility.	Combination of the enzyme and substrate.
Combination of enzyme with products.	Positive autocatalysis.
Negative autocatalysis.	
Destruction or change in the enzyme.	

**Reversibility of Enzyme Action.** The reversibility of enzyme action has been proved in other cases. Emmerling<sup>2</sup> has shown that amygdalin is formed when yeast maltase is added to a solution of glucose and mandelonitrile glucoside (p. 75), and Kastle and Loevenhart<sup>3</sup> have obtained ethyl butyrate by the action of lipase on ethyl alcohol and butyric acid, that is, by the enzyme which produces the two latter products by hydrolysis.

The same enzyme, which also hydrolyses fats, can effect the synthesis of butyric and mono- and tri-oleins from glycerol and the fatty acids,<sup>4</sup> whilst van 't Hoff<sup>5</sup> and Bayliss<sup>6</sup> have obtained glycerol  $\beta$ -glucoside by the action of emulsin on a mixture of glycerol and glucose, and Bourquelot and Bridel and other co-workers<sup>7</sup> have prepared a whole series of crystalline  $\alpha$ - and  $\beta$ -alkyl glucosides, using maltase and emulsin and a variety of alcohols. Indications have also been obtained that trypsin, pepsin, and crepsin can produce proteins from the same amino acids which they form by hydrolysis.<sup>8</sup>

These facts would fit in very well with the idea of the catalytic action of the enzyme in a reversible process, seeing that the equilibrium-point is usually unchanged by different concentrations of enzyme or by enzymes obtained from different preparations.

There are certain facts, however, which appear to be opposed to this view. That the equilibrium-point should be different in the case of enzymes from that produced by mineral acids may be explained by

<sup>1</sup> *Trans. Chem. Soc.*, 1898, 73, 634.

<sup>2</sup> *Ber.*, 1901, 34, 3810.

<sup>3</sup> *Amer. Chem. J.*, 1900, 24, 491.

<sup>4</sup> Hanriot, *Compt. rend.*, 1901, 132, 212.

<sup>5</sup> *Sitz. Ber. K. Preuss. Akad.*, 1910, 48, 963.

<sup>6</sup> *Journ. Physiol.*, 1913, 46, 236.

<sup>7</sup> *Ann. Chim. Phys.*, 1913, 28, 145; *Journ. Pharm.*, 1914, 10, 361; *Compt. rend.*, 1917, 164, 443, 521, 831; 1918, 168, 253, 1016.

<sup>8</sup> Abderhalden, *Chem. Zentr.*, 1915, i, 899; Taylor, *J. Biol. Chem.*, 1907, 3, 87.

the different mechanism of enzyme action in which the enzyme, being a colloid, is in a different phase from that of the substrate, and acts probably, in the first instance, by adsorption. The statement laid down by Hudson,<sup>1</sup> that the hydrolysis of sucrose by invertase is complete and apparently non-reversible, merely implies that the balance has been pushed to a limit in one direction, and is not necessarily opposed to the theory of catalytic action. But there are more difficult problems requiring solution.

Yeast extract (which contains maltase) acting on glucose is said to produce, in addition to small quantities of maltose, mainly isomaltose, which is a  $\beta$ -glucoside and can only be hydrolysed by emulsin or by ordinary brewers' yeast (p. 72). Similar results have been obtained in the synthesis of isolactose by the action of kephir lactase on a mixture of glucose and galactose (p. 73). Moreover, Rosenthaler<sup>2</sup> claims to have separated from emulsin at least two enzymes, of which only one hydrolyses mandelonitrile whilst the other synthesises it as well.

Bayliss proposes by way of explanation that in the synthesis of isomaltose, which is formed with maltose, the former may be produced by the action of emulsin, which is now known to be present in brewers' yeast<sup>3</sup> or possibly, as Fajan suggests, the same enzyme may act so as to produce both isomers but at different rates.<sup>4</sup> This is certainly true of lipase, which, as Dakin has shown, hydrolyses both esters of enantiomorphous acids, but with different velocities (see p. 81), so that, unless the hydrolysis is complete, an excess of one enantiomorph will be decomposed and an active acid produced.

In regard to Rosenthaler's results, Bayliss considers that the experimental evidence needs verification.

It follows from what has been stated that the same enzyme may manifest a different activity according to whether it attacks one or other group of atoms in the substrate. On the other hand, the view that the difference in specific action is always traceable to separate enzymes, though affording a simple way out of an immediate difficulty, may ultimately produce problems of an even more involved kind and possibly more difficult of interpretation. Bayliss is inclined to the view that the notion of specific action has been pushed too far, and that there may be, in addition to keys fitting special locks, 'master-keys' as well, which, like lipase, can react with both active forms but at different rates. If such is the case, the problem can best be solved

<sup>1</sup> *J. Amer. Chem. Soc.*, 1914, **36**, 1571.

<sup>2</sup> *Biochem. Zeitsch.*, 1908, **14**, 238; 1909, **17**, 257.

<sup>3</sup> Henry and Auld, *Proc. Roy. Soc.*, 1905, **76 B**, 568.

<sup>4</sup> *Zeit. physik. Chem.*, 1910, **73**, 25.

by the study of the dynamics of enzyme action, when it may be found that various so-called specific enzymes may merely imply degrees of hydrolytic or synthetic activity, determined by the nature of the enzyme and its environment.

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## CHAPTER III

### THE PURINE GROUP

**Historical.** The history of the group of compounds which forms the subject of the present chapter has its origin in those curious pathological concretions which are found in the human body, as chalk-stones and urinary calculi. The composition of these substances attracted the attention of the early physicians and chemists, who speculated freely on their origin without discovering much about their chemical nature. It is curious to find that Paracelsus regarded them as having a similar origin to the lees of wine, and named them *tartur*.<sup>1</sup> The discovery in urinary calculi of uric acid, or as it was then termed *lithic acid*, is due to Scheele, who isolated the acid in 1766 and observed at the same time the red colour which it gives on evaporation with nitric acid. Prout<sup>2</sup> afterwards noticed that ammonia changes the colour to violet, forming *muresside*, a reaction which is still used as a delicate test for the acid. In 1798 Pearson found uric acid in the deposit which sometimes forms in the tissues of persons suffering from gout, and since then its occurrence has been observed in many different parts of the body. It is found in variable amount in the urine of most animals, and constitutes the bulk of the excreta of birds and reptiles. It is an interesting fact that the white colour on the wings of butterflies, known as *White Pieridae*, is due to uric acid.

**Decomposition Products of Uric Acid.** In 1793 Fourcroy observed the formation of urea when chlorine water acts upon uric acid; a little later Brugnatelli obtained a crystalline compound, now known as *alloxan*, by the action of nitric acid; its elementary composition,  $C_5H_4N_4O_3$ , was determined in 1834 by Liebig and Mitscherlich. But the first serious contribution to our knowledge of uric acid is contained in a remarkable memoir by Wöhler and Liebig,<sup>3</sup> entitled 'Untersuchungen über die Natur der Harnsäure', which was published

<sup>1</sup> *Treatise on Chemistry*, by Roscoe and Schorlemmer, vol. iii, part ii, p. 331.

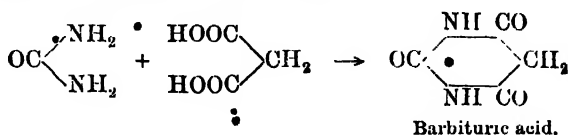
<sup>2</sup> *Phil. Trans.*, 1818, p. 420.

<sup>3</sup> The modern formulæ are used.

<sup>4</sup> *Annalen*. 1838, 26, 241.

in 1838, and still serves as a model of scientific acumen and experimental skill. They showed how uric acid by regulated oxidation could be converted into a series of substances of diminishing complexity. Thus, by direct oxidation they obtained the three compounds, *allantoin*  $C_4H_6N_4O_3$ , *alloxan*  $C_4H_2N_2O_4$ , and *parabanic acid*  $C_3H_2N_2O_3$ . From alloxan they prepared by reduction *alloxantin*,  $C_3H_6N_4O_3$ , and *dialuric acid*,  $C_4H_4N_2O_4$ ; by the action of ammonium sulphite, *thionuric acid*,  $C_4H_5N_3SO_6$ , and from the latter, by boiling with dilute mineral acids, *uramil*,  $C_4H_5N_3O_3$ . To this collection of what may be termed the fragments of the uric acid molecule, Schlieper<sup>1</sup> afterwards added several new compounds. As alloxan was found to undergo hydrolysis into mesoxalic acid and urea, Gerhardt suggested that its formula should be represented as mesoxalyl urea. But apart from this, little was known about the constitution of these various derivatives until the years 1863 and 1864, when Baeyer,<sup>2</sup> in a series of masterly researches, placed the whole subject in a clear light, and so prepared the way for the subsequent discovery of the structure and synthesis of uric acid and of the allied xanthine bases.

Without entering into detail, the manner in which Baeyer arrived at some of his results may be briefly indicated. Dialuric acid, obtained by reducing alloxan, is converted, on heating, into *hydrilic acid*, which may be regarded as an anhydride of alloxan and barbituric acid (see below). With nitrous acid, hydrilic acid is partly converted into *violuric acid*, and with nitric acid into *dilituric acid*. Both violuric acid and dilituric acid, on reduction, form uramil, and with bromine they yield the same product, which Baeyer first termed alloxan bromide, but afterwards altered to *dibromo-barbituric acid*. In the first case an isonitroso group, in the second a nitro group and a hydrogen atom are exchanged for two bromine atoms. Dibromo-barbituric acid on reduction forms *barbituric acid*. Now, since barbituric acid is decomposed with potash into urea and malonic acid, it is probably malonyl urea, a view which was afterwards confirmed by Grimaux,<sup>3</sup> who synthesised it by heating together malonic acid, urea, and phosphorus oxychloride :

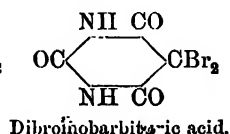
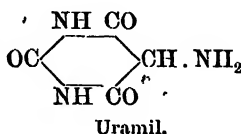
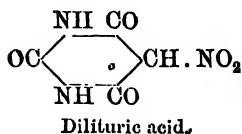
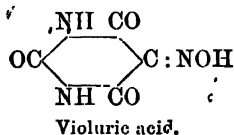
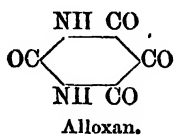
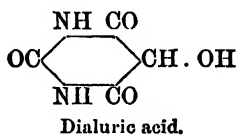


<sup>1</sup> *Annalen*, 1845, 56, 1.

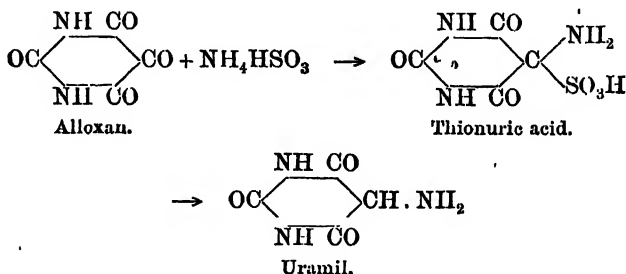
<sup>2</sup> *Annalen*, 1863, 127, 1, 199; 1864, 130, 129; 131, 291.

<sup>3</sup> *Bull. Soc. Chim.*, 1876, 31, 146.

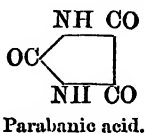
Baeyer represented the relation in which the compounds, referred to above, stand to barbituric acid by the following formulae:



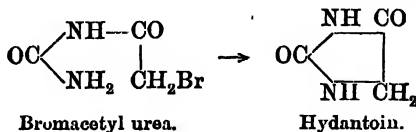
The constitution of thionuric acid is determined by its formation from alloxan and ammonium sulphite and its conversion into uramil.



The structure of parabanic acid is derived from its resolution into urea and oxalic acid, and its subsequent synthesis by Grimaux from these two substances with the aid of phosphorus oxychloride.



Hydantoin, or glycolyl-urea, which is formed by the reduction of allantoin, was synthesised by the action of ammonia on bromacetyl-urea by Baeyer, who thus prepared the first synthetical ureide.



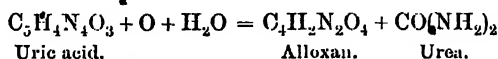
Baeyer divides the above compounds into two classes, the paraban series and the alloxan series.<sup>1</sup>

Paraban Series.	Alloxan Series.	
Parabanic acid (Oxalyl-urea)	Alloxan (Mesoxalyl-urea)	Violuric acid
Oxaluric acid	Dialuric acid (Tartronyl-urea)	Dilituric acid
Hydantoin (Glycolyl-urea)	Barbituric acid (Malonyl-urea)	Thionuric acid
Allanturic acid (Glyoxyl-urea)	Dibromo-barbituric acid	Uramil

In addition to the above, there is a series of diureides, which may be regarded as combinations of two molecules of certain of the above compounds. The exact nature of this union is not definitely known in every case.

Diureide.	Constituent molecules.	Probable formula.
Allantoin	Hydantoin + Urea	$\begin{array}{c} \text{NH}_2 \quad \text{OC} \quad \text{NH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{OC} \quad \text{NH} - \text{CH} - \text{NH} \quad \text{CO} \end{array}$
Hydurillic acid <sup>2</sup>	Alloxan + Barbituric acid	$\begin{array}{c} \text{NH} \quad \text{CO} \quad \text{CO} \quad \text{NH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{OC} \quad \text{NH} - \text{CH} - \text{HC} \quad \text{CO} \quad \text{NH} \quad \text{CO} \end{array}$
Alloxantin	Alloxan + Dialuric acid	$\begin{array}{c} \text{NH} \quad \text{CO} \quad \text{HO} \cdot \text{C} \quad \text{NH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{OC} \quad \text{NH} - \text{C}(\text{OH}) \cdot \text{O} \cdot \text{C} \quad \text{CO} \quad \text{NH} \quad \text{CO} \end{array}$
Murexide <sup>3</sup>	Alloxantin + Ammonia	$\begin{array}{c} \text{NH} \quad \text{CO} \quad \text{CO} \quad \text{NH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{OC} \quad \text{NH} - \text{C} \quad \text{N} \quad \text{C} \quad \text{CO} \quad \text{NH} \quad \text{CO} \end{array}$
Violantin	Violuric acid + Dilituric acid	—

**Structure of Uric Acid.** As uric acid is found to break up on oxidation into equal molecules of alloxan and urea according to the equation:



it would appear to be a compound of alloxan and urea, in which the two components are united with the loss of two hydroxyl groups.

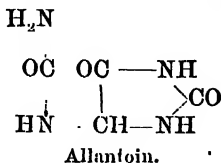
<sup>1</sup> For the special properties of the individual compounds a book of reference must be consulted.

<sup>2</sup> Conrad, *Annalen*, 1907, 356, 24; Biltz and Hamburger, *Ber.*, 1916, 49, 655; 1919, 52, 1298.

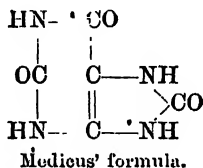
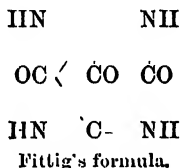
<sup>3</sup> Mohlau, *Ber.*, 1904, 37, 286; Piloty and Finckh, *Annalen*, 1904, 333, p. 22.



If the oxidation is carried out with neutral or alkaline permanganate, or with lead peroxide, the reaction takes a different course and allantoin is formed. Allantoin is a diureide, whose structure is known from its direct synthesis from glyoxalic acid and urea.<sup>1</sup>



The presence of four imino groups in uric acid agrees with the existence of a tetramethyluric acid, obtained by the direct methylation of uric acid, from which all the nitrogen can be removed as methylamine on heating with strong hydrochloric acid. These facts have found expression in two formulæ for uric acid, one proposed by Fittig<sup>2</sup> in his text-book, and the other by Medicus:<sup>3</sup>



Fittig's formula represents a symmetrical arrangement of two condensed pyrimidine nuclei, that of Medicus contains a fused pyrimidine and iminazole ring.

A clear indication of the correctness of Medicus' formula as opposed to that of Fittig was afforded by Fischer's<sup>4</sup> discovery of a second monomethyluric acid, in addition to the one previously described by Hill,<sup>5</sup> both of which are formed simultaneously by treating the lead salt of uric acid with methyl iodide. Since one of these compounds gives, on oxidation, methyl alloxan and urea, and the other, by similar treatment, alloxan and methyl urea, the formula of uric

<sup>1</sup> Grimaux, *Ann. Chim. Phys.*, 1877 (5), 11, 389.

It has been shown that allantoin is not a direct product of the oxidation of uric acid. It is probable that both of the ring systems in uric acid are broken on oxidation and that the allantoin is formed as the result of complicated intramolecular changes. This observation diminishes the value of the evidence afforded by the formation of allantoin. Although allantoin contains an asymmetric carbon atom, the natural product obtained from dogs' urine is inactive. This has been shown to arise from isomeric change in which the hydrogen atom attached to the asymmetric carbon wanders to the neighbouring oxygen. Dakin, *Amer. Chem. J.*, 1910, 44, 48.

<sup>2</sup> *Grundriss der organischen Chemie.*

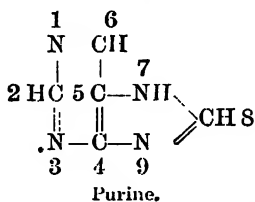
<sup>3</sup> *Ber.*, 1884, 17, 1777.

<sup>4</sup> *Annalen*, 1875, 175, 236.

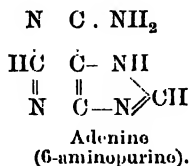
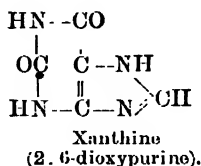
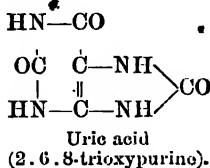
<sup>5</sup> *Iber*, 1876, 9, 370, 1090.

acid must be represented by the fusion of an alloxan and a urea nucleus, so as to form an unsymmetrical grouping as proposed by Medicus. According to Fittig's formula only one monomethyl uric acid should exist. The subsequent preparation of all the theoretically possible methyluric acids as well as the various syntheses of uric acid, to which reference will presently be made, have in every case confirmed the original formula assigned by Medicus, which is now universally accepted.

**Nomenclature.** Fischer has shown that the same atomic framework is present in uric acid and the numerous xanthine bases and denotes the relative position of the atoms by the numbers 1 to 9. If the four additional hydrogen atoms necessary to satisfy the valencies of the carbon and nitrogen atoms be added, the structure of the parent substance of the series is obtained, a compound which has been prepared by Fischer and named *purine* (*purum uricum*). This substance will be referred to later (p. 123).

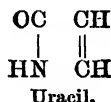


- Uric acid and the xanthine bases may be regarded as substituted purines. As an illustration of the above system of nomenclature, uric acid will appear as 2.6.8-trioxypurine, whilst xanthine and adenine (p. 123) are 2.6-dioxypurine and 6-aminopurine respectively.



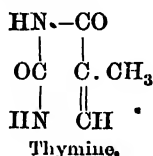
- Uracil and its Derivatives.** Before discussing the synthesis of uric acid reference must be made to a group of uracils which, like those already mentioned, appear to play an interesting rôle in animal metabolism.<sup>1</sup> The name *uracil* was given by Behrend to the simplest member of this special group.

<sup>1</sup> Burian has recently demonstrated the formation of uracil derivatives from purine bases, when the latter are treated with strong sulphuric acid in the presence of sugar.

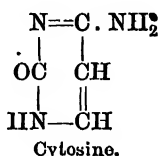


Uracil.

Though the compound itself has only recently been prepared, several of its derivatives have long been known and were isolated by Kossel and his pupils from the products of hydrolysis of nucleic acids (p. 165). Among these are *thymine* (5-methyl-uracil<sup>1</sup>) and *cytosine* (6-amino-uracil).

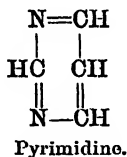


Thymine.



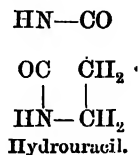
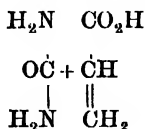
Cytosine.

These substances may be regarded as oxy-derivatives of the parent ring compound, *pyrimidine*.

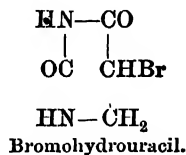


Pyrimidine.

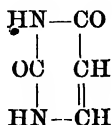
Uracil itself was first prepared by Fischer and Roeder<sup>2</sup> by heating urea and acrylic acid to 210°, and the product, *hydrouracil*, was then brominated. When treated with pyridine, bromohydrouracil loses hydrogen bromide and gives uracil.



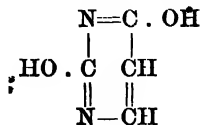
Hydrouracil.



Bromohydrouracil.



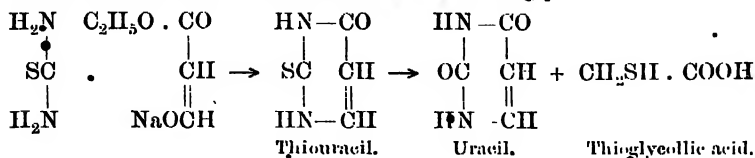
Uracil.



<sup>1</sup> The numbering of the atoms in uracil derivatives follows the order adopted in the case of uric acid.

<sup>2</sup> Ber., 1901, 34, 3751.

A more convenient method of obtaining it is described by Wheeler and Middle,<sup>1</sup> and consists in condensing thiourea with sodium formylacetic ester and then exchanging the sulphur for oxygen by means of chloroacetic acid, which forms uracil and thioglycollic acid.

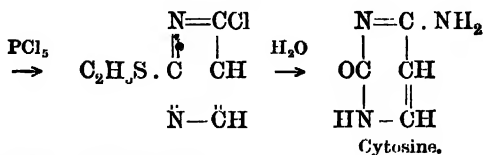
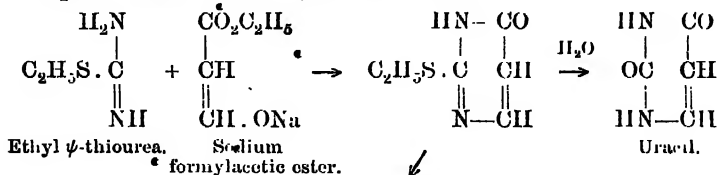


Both thymine and cytosine have also been synthesised, the former by Behrend from a methyl acrylic acid by a series of reactions similar to those used in Fischer's synthesis of uracil, and both thymine and cytosine by Wheeler and Johnson,<sup>2</sup> who by an important modification of Fischer's and Behrend's method have added a very large number of new members to the list of pyrimidine derivatives.

In place of urea they substitute an alkyl  $\psi$ -thiourea or thiourea, which are much more reactive, and replace acrylic acid and its derivatives by a variety of aldehydic and ketonic esters, in short, by compounds which in presence of alkali can function in the tautomeric form as hydroxy- or ethoxy-acrylic esters.

The following may be taken as a typical example of the method:

Ethyl  $\psi$ -thiourea and sodium formylacetic ester condense in aqueous solution with the formation of 2-ethylmercapto-6-oxypyrimidine, which, on the one hand, by boiling with hydrobromic acid, breaks up into ethyl mercaptan and uracil, and, on the other, when treated with phosphorus pentachloride yields a chlorine derivative. By the action of alcoholic ammonia on the latter the chlorine is replaced by the amino group, and, on hydrolysis with hydrobromic acid as before, mercaptan is removed and cytosine is formed.

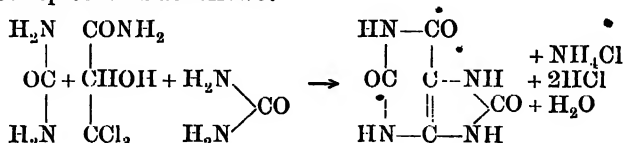


<sup>1</sup> *Amer. Chem. J.*, 1905, 40, 547.

<sup>2</sup> *Amer. Chem. J.*, 1903, 29, 478; 1909, 42, 353; 1910, 43.

In precisely the same way acetoacetic ester gives 4-methyl-uracil; formylpropionic ester gives 5-methyl-uracil (thymine), and methyl acetoacetic ester forms dimethyl-uracil, and each of these in turn may be converted into the corresponding cytosine derivative. Carboxyl derivatives have also been prepared by replacing the hydroxyacrylic ester by ethoxymethylene malonic ester and formyl succinic ester. The pyrimidine compounds obtained in this way are very reactive; the hydrogen atoms attached to carbon 4 and 5 atoms may be replaced by bromine, that of carbon 5 by a nitro group; the mercaptol group in the original condensation product may be replaced by aromatic basic radicals, the chlorine of carbon atom 6 by amino groups, and isothiocyanate radicals, the hydrogen of the  $\text{NH}$  groups 1 and 3, by benzyl and other alkyl and acid radicals, by the action of alkyl and acyl halide in presence of alkali. It will be readily understood how great a variety of pyrimidine derivatives may be obtained by combining these reactions, which are too numerous to record in detail in this volume.<sup>1</sup>

**Synthesis of Uric Acid.** *Horbaczewski's Synthesis.* In 1868 Strecker<sup>2</sup> found that uric acid could be hydrolysed at high temperatures with the production of ammonia, carbon dioxide, and glyccoll. Horbaczewski<sup>3</sup> some years later applied the fact to the synthesis of uric acid, which he effected by heating urea with glyccoll or cyanacetic acid. The yield, however, is extremely small, and the reactions are so complex that the synthesis throws little light upon the structure of uric acid, and from this point of view is of no value. Horbaczewski showed later that a somewhat better result is obtained by heating the amide of trichlorolactic acid with urea. The reaction may be represented as follows:



*Behrend and Roosen's Synthesis.* Another synthesis was accomplished a little later by Behrend and Roosen, which, though much less direct than the preceding, conveys more information about the structure of the compound in question. Behrend and Roosen<sup>4</sup>

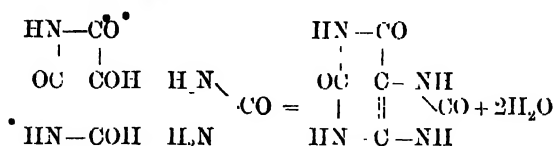
<sup>1</sup> A list of researches on pyrimidine compounds is given in the *Amer. Chem. J.*, 1908, 40, 250; but a large number of papers have appeared in the same journal since that date by Wheeler, Johnson, Johns, and their collaborators.

<sup>2</sup> *Annalen*, 1868, 146, 142.

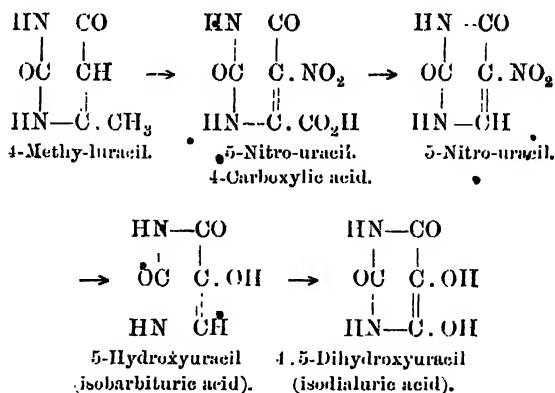
<sup>3</sup> *Ber.*, 1882, 15, 2678; *Monatsh.*, 1885, 6, 356; 1887, 8, 201, 584.

<sup>4</sup> *Ber.*, 1888, 21, 999; *Annalen*, 1888, 251, 235.

showed that *isodialuric acid* undergoes condensation with urea, in the presence of strong sulphuric acid, to form uric acid.\*



The preparation of isodialuric acid is a long and complicated process. The starting-point is 4-methyl-uracil, which results from the saponification of the condensation product obtained by the interaction of acetoacetic ester and urea. The methyl-uracil is first treated with nitric acid, a process which not only introduces a nitro group, but at the same time oxidises the methyl to a carboxyl group. The acid thus formed readily loses carbon dioxide on boiling with water, and changes into 5-nitro-uracil. This nitro derivative, on reduction with tin and hydrochloric acid, yields both 5-amino-uracil and 5-hydroxyuracil (isobarbituric acid), and the latter substance, on oxidation with bromine water, forms isodialuric acid.



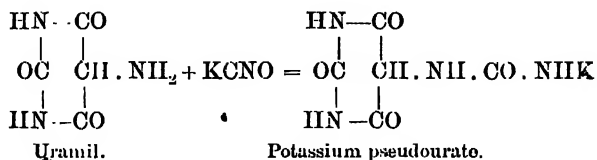
*F. Fischer's Synthesis.* The starting-point of Fischer's synthesis<sup>1</sup> is pseudouric acid, the compound which many years before Liebig and Wöhler<sup>2</sup> attempted in vain to prepare with the object of converting it into uric acid. Baeyer and Schlieper<sup>3</sup> were more successful, and

<sup>1</sup> Ber., 1897, 30, 559.

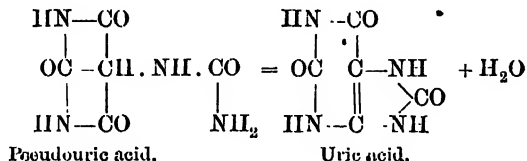
<sup>2</sup> Annalen, 1838, 26, 241.

<sup>3</sup> Annalen, 1863, 127, 3.

obtained it in the form of the potassium salt by boiling uramil with a solution of potassium cyanate, from which pseudouric acid was then liberated.

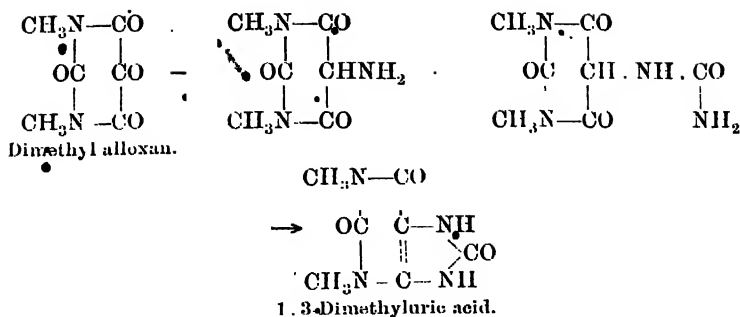


Pseudouric acid differs in composition from uric acid by one molecule of water, but the early attempts to remove this molecule of water and convert pseudouric into uric acid, failed. In Fischer's hands the operation was not only successful, but the process has served with little modification for the synthesis of other members of the purine group. The method which Fischer adopted was to boil pseudouric acid with hydrochloric acid.

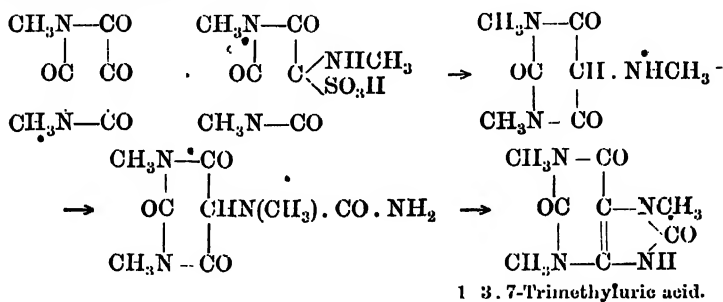


The synthesis involves the preparation of uramil, which, as we have seen, may be obtained from barbituric acid through violaric acid and also from alloxan through thionuric acid (p. 104). But the production of barbituric acid necessitates that of malonic acid, whilst alloxan is derived from barbituric acid or from uric acid itself. Thus, the synthesis depends in the first instance on the production of malonic acid.

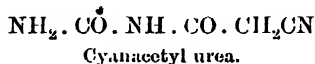
The above synthetic method has been successfully applied by Fischer to the production of various alkyl derivatives of uric acid and, indirectly, to that of many of the xanthine bases, such as theobromine, theophylline, caffeine, &c. For example, mono- and di-alkyluric acids have been prepared from mono- and di-alkyl alloxans, which are converted into the corresponding uramils, pseudouric and uric acids. Thus, dimethyl alloxan yields 1.3-dimethyluric acid.



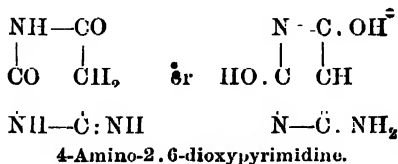
Moreover, it is possible to introduce a methyl group into position 7 by using methylamine sulphite in place of ammonium sulphite. 1, 3, 7-Trimethyluric acid (hydroxycaffeine) may be synthesised from dimethyl alloxan in this way:



*W. Traube's Synthesis.* Another synthetic method for the preparation of uric acid is described by W. Traube.<sup>1</sup> In this case the starting point is either cyanacetic acid or its ester. Cyanacetic acid and urea in presence of phosphorus oxychloride form cyanacetyl urea,



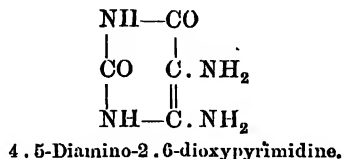
which by the action of alkalis is converted into 4-amino 2, 6-dioxypyrimidine.



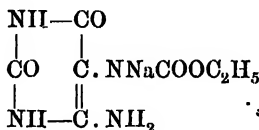
<sup>1</sup> *Ber.*, 1900, 33, 1871, 3035.



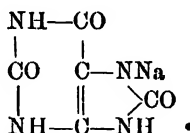
Nitrous acid then replaces the hydrogen of the methylene group by an isonitroso group, and the latter, on reduction with ammonium sulphide, is converted into an amino group, yielding the following compound:



By the action of chloroformic ester a urethane is produced, which forms a sodium salt.



When the sodium salt is heated, alcohol is eliminated and sodium urate is formed.



A similar method has been applied to the synthesis of xanthine, guanine, and similar compounds, to which reference is made on p. 124.

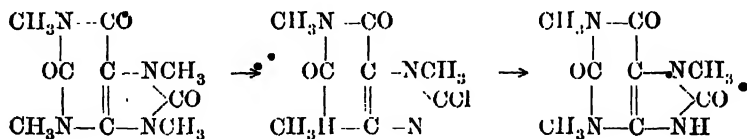
**The Alkyluric Acids.** In addition to the methyluric acids which may be prepared by Fischer's synthetic method already described, a number of these compounds have been obtained by the direct methylation of uric acid. The process is carried out by heating the lead or silver salt with methyl iodide, or, more conveniently, by shaking up uric acid with methyl iodide or methyl sulphate<sup>1</sup> in the presence of dilute caustic soda. The positions which the methyl groups assume depend to some extent upon the method of methyla-

<sup>1</sup> Billz and Damm, *Annalen*, 1916, 413, 186.

tion and the temperature employed. The following methyluric acids have been obtained by the direct methylation of uric acid<sup>1</sup>:

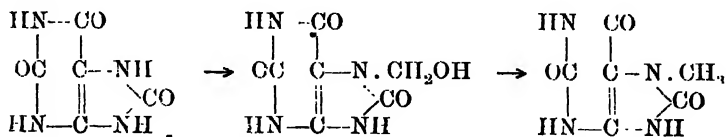
3-Methyluric acid.	• 1. 3-Dimethyluric acid.	1. 3. 7-Trimethyluric acid.
9-Methyluric acid.	3. 9-Dimethyluric acid.	3. 7. 9-Trimethyluric acid.
	7. 9-Dimethyluric acid.	1. 3. 7. 9-Tetramethyluric acid.

By the methylation of alkyluric acids of known constitution, prepared synthetically by Fischer's method, it has been possible to prepare some additional methyluric acids. In some cases a process of *demethylation* has been successfully employed. For example, if tetramethyluric acid—the product of the complete methylation of uric acid—be heated with phosphorus oxychloride, the methyl group in position 9 is detached, and at the same time the oxygen atom in position 8 is replaced by chlorine.



The 1. 3. 7-trimethyl-8-chloropurine produced in this way can be converted into 1. 3. 7-trimethyluric acid by alkalis, or may be used directly for other syntheses (cp. synthesis of caffeine, p. 121).

• An additional method of preparation of methyluric acid consists in the reduction of the hydroxymethylene derivatives of uric acid, which are produced by the action of formaldehyde upon alkaline urates. For example, 7-methyluric acid is formed by the reduction of the compound produced by the interaction of formaldehyde and uric acid<sup>2</sup>:

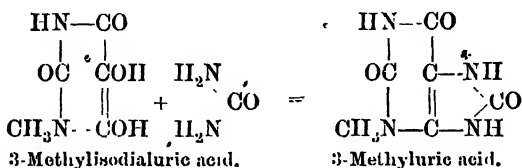


• It was supposed that, in addition to the four isomeric monomethyluric acids, obtained by the replacement of each of the four hydrogen atoms of the imino groups in uric acid, two other mono-

<sup>1</sup> Reference to the method of preparation and structure of all the methyluric acids will be found in Fischer's paper, *Ber.*, 1899, 32, 461.

<sup>2</sup> *Chemisches Centralblatt*, 1900, 6, p. 270.

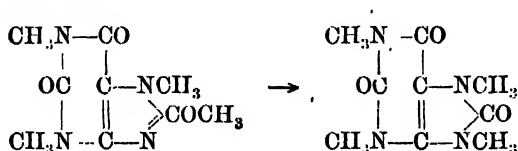
methyluric acids existed, in both of which the methyl group is attached to nitrogen in position 3, making in all three 3-methyluric acids. They were known respectively as the  $\alpha$ -,  $\delta$ -, and  $\zeta$ -methyluric acids. The  $\alpha$ - and  $\zeta$ -acids are prepared by the direct methylation of uric acid, the former by the usual method of alkylation, the latter by methylation in the presence of acetic acid.  $\delta$ -Methyluric acid was obtained by v. Loeben<sup>1</sup> from 3-methylisodialuric acid by a similar method to that employed by Behrend and Roosen in their synthesis of uric acid (p. 110).



According to Biltz and Heyn<sup>2</sup> the  $\alpha$ - and  $\zeta$ -acids are mixtures containing, in addition to the  $\delta$ -acid, 25 and 10 per cent. respectively of 9-methyluric acid, v. Loeben's  $\delta$ -acid being the pure 3-methyluric acid.

Isomerism is found in the case of tetramethyluric acid and methoxycaffeine. The first is obtained by the action of methyl iodide on 1.3.7-trimethyluric acid in presence of an alkali, the second by its action on the silver salt of the acid, whilst both compounds are produced by the direct methylation of  $\zeta$ -methyluric acid.

W. Wislicenus and Körber<sup>3</sup> have shown that methoxycaffeine passes on heating into tetramethyluric acid, a change which they represent as follows:



**The Structure of the Xanthine Bases.** One of the most notable developments in the history of the purine compounds belongs to the year 1881, when Emil Fischer<sup>4</sup> published his investigation on caffeine, theobromine, xanthine, and guanine, a memoir which still ranks as one of the many brilliant achievements of this distinguished

<sup>1</sup> v. Loeben, *Annalen*, 1897, 298, 181.

<sup>2</sup> *Annalen*, 1916, 413, 98.

<sup>3</sup> *Ber.*, 1902, 35, 1901.

<sup>4</sup> *Ber.*, 1881, 14, 637, 1905; 1882, 15, 29; *Annalen*, 1882, 215, 253.

chemist. Xanthine is mainly a product of the animal organism and occurs combined in some nucleic acids, though it has also been detected in plant seedlings; theobromine is a constituent of cocoa beans (*theobroma cacao*); caffeine occurs in small quantities in tea and coffee; and guanine is associated with uric acid in guano and is a constituent of certain nucleic acids. Although of widely different origin, the close chemical relationship existing between them and uric acid had long been suspected. The composition and properties of xanthine and guanine are intimately related to those of uric acid.

Uric Acid  $C_5H_4N_4O_3$

Xanthine  $C_5H_4N_4O_2$

Guanine  $C_5H_5N_5O$

Stenhouse<sup>1</sup> observed a reaction with caffeine which closely resembled the murexide test, whilst Strecker<sup>2</sup> succeeded in converting guanine into xanthine by the action of nitrous acid. In a subsequent paper Strecker<sup>3</sup> explained the relationship of caffeine, theobromine, and xanthine by representing caffeine as trimethylxanthine and theobromine as dimethylxanthine. Though unsuccessful in methylating xanthine, he so far confirmed his views as to convert theobromine into caffeine by the action of methyl iodide upon the silver salt. So much was known when Fischer<sup>4</sup> began his investigations. His first attack was directed against the caffeine molecule, which, as he anticipated, yielded more readily than the other compounds to the disintegrating action of reagents.

Caffeine,  $C_8H_{10}N_4O_2$ , on oxidation with chlorine water breaks up into equal molecules of dimethylalloxan and monomethylurea, and thus nine of the ten hydrogen atoms are present as methyl groups. With chlorine and bromine caffeine yields a monochloro- and monobromo-substitution product. By the action of alcoholic ammonia on the latter, the halogen is exchanged for an amino group; with alcoholic potash, for an ethoxyl group. The latter, on warming with dilute hydrochloric acid, yields hydroxycaffeine, which has since been identified as 1.3.7-trimethyluric acid. Hydroxycaffeine forms an additive compound with two atoms of bromine, yielding a dibromohydroxycaffeine, in which the bromine can be replaced by two ethoxyl groups by means of alcohol. The following formulae indicate the relation of the above series of compounds:

<sup>1</sup> *Annalen*, 1833, 45, 366; 46, 227.

<sup>3</sup> *Annalen*, 1861, 118, 170.

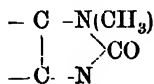
<sup>2</sup> *Annalen*, 1859, 108, 141.

<sup>4</sup> *Annalen*, 1882, 215, 253.

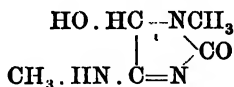
Caffeine	$\text{CH}_7(\text{NCH}_3)_3\text{O}_2\text{N}$
Chlorocaffeine	$\text{C}_5\text{Cl}(\text{NCH}_3)_3\text{O}_2\text{N}$
Aminocaffeine	$\text{C}_5(\text{NH}_2)(\text{NCH}_3)_3\text{O}_2\text{N}$
Hydroxycaffeine	$\text{C}_5(\text{OH})(\text{NCH}_3)_3\text{O}_2\text{N}$
Dibromohydroxycaffeine	$\text{C}_5(\text{OH})(\text{NCH}_3)_3\text{O}_2\text{NBr}_2$
Diethoxyhydroxycaffeine	$\text{C}_5(\text{OH})(\text{NCH}_3)_3\text{O}_2\text{N}(\text{OC}_2\text{H}_5)_2$

As the constitution of caffeine has been recently established by a simple and direct synthesis, and received in consequence a slightly different interpretation from the one originally attached to it by Fischer, the nature of the degradation products of the caffeine molecule, upon which the structure formerly depended, has lost something of its interest and value. The subject will therefore be treated quite shortly.

The decomposition of caffeine into dimethylalloxan and methyl urea indicates an atomic framework similar to that of uric acid. It also determines the positions of two methyl groups and one oxygen atom, and there is probably a double bond. Positions have therefore to be assigned to the third methyl group, one oxygen and one hydrogen atom. When diethoxyhydroxy-caffeine is boiled with hydrochloric acid it breaks up into methylamine, alcohol, and *apocaffeine*. Boiled with water, *apocaffeine* is in turn resolved into carbon dioxide and *caffuric acid*, and finally, *caffuric acid* can be hydrolysed into mesoxalic acid, methylamine, and methyl urea. By the action of hydrogen iodide on *caffuric acid*, *hydrocaffuric acid* is formed, which breaks up, on boiling with baryta, into methylamine, carbon dioxide, and methylhydantoin. Methylhydantoin contains the following atomic skeleton:

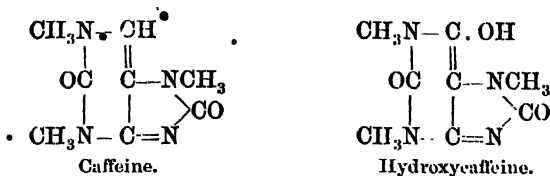


By the action of hydrochloric acid on diethoxyhydroxycaffeine, in addition to *apocaffeine*, *hypocaffeine* is formed, which is decomposed by bases into *caffoline* and carbon dioxide. As *caffoline* is converted on oxidation into dimethyloxamide, it probably possesses the following formula:

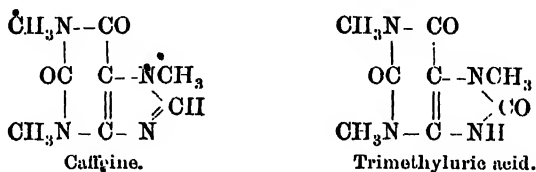


Assuming methylhydantoin and *caffoline* to represent the iminazole nucleus, an assumption which is justified by the relative

positions of the methylamino groups, Fischer represented caffeine and hydroxycaffeine by the following formulæ:



The subsequent discovery that hydroxycaffeine was identical with trimethyluric acid, and yielded by further methylation with methyl iodide and potash, tetramethyluric acid, led to the adoption of the formula proposed by Medicus, for it is clear that tetramethyluric acid could not be derived from a substance possessing the formula originally assigned by Fischer.

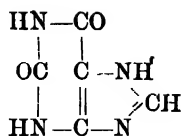


Fischer observed that xanthine, on oxidation with potassium chlorate and hydrochloric acid, was converted into alloxan and urea, whilst, on heating xanthine with hydrochloric acid at 190°, it was resolved into glycocoll, ammonia, and carbon dioxide. Both of these reactions clearly established the close relation existing between xanthine and uric acid, and the further observation that theobromine was formed by the action of methyl iodide upon the lead salt of xanthine established its connection with theobromine and caffeine.

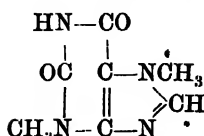
The constitution of theobromine is deduced from its conversion into caffeine on methylation, and by the fact that it yields methylalloxan and methylurea upon oxidation; further, that bromotheobromine is converted with potash into hydroxytheobromine, which has been identified as 3.7-dimethyluric acid.

Strecker's earlier discovery, that guanine can be transformed by nitrous acid into xanthine on the one hand and oxidised to guanidine on the other, determined the structure of guanine as an amino xanthine. The accepted formulæ of these compounds are those originally assigned to them by Medicus.<sup>1</sup>

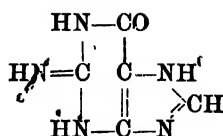
<sup>1</sup> *Annalen*, 1875, 175, 236.



Xanthine.



Theobromine.



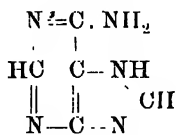
Guanine.

The above are not the only xanthine derivatives which occur in nature. Since Fischer's paper appeared new methyl xanthines have been brought to light. The following table contains a list of these compounds, their sources and their structure. Those which occur in urine are probably derived from the breaking down of caffeine.

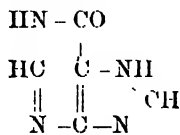
*Natural Xanthine Bases.*

Name.	Synonym.	Occurrence.
Xanthine	--	Animal tissues
1 Methylxanthine	--	Urine
7-Methylxanthine	Heteroxanthine	Urine
1.3 Dimethylxanthine	Theophylline	Tea
1.7-Dimethylxanthine	Paraxanthine	Urine
3.7-Dimethylxanthine	Theobromine	Cocoa
1.3.7-Trimethylxanthine	Caffeine	Tea, coffee, kola, &c.

In addition to the above, *hypoxanthine* and *adenine*, though not strictly xanthine derivatives, may be referred to as accompanying guanine and xanthine in the products of hydrolysis of nucleic acids. They are closely related to one another, for adenine is converted into hypoxanthine by the action of nitrous acid. Hypoxanthine is 6-oxypurine, and adenine, the corresponding amino-derivative.



Adenine.

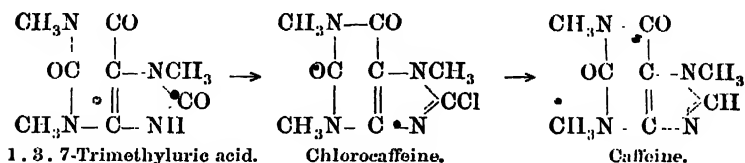


Hypoxanthine.

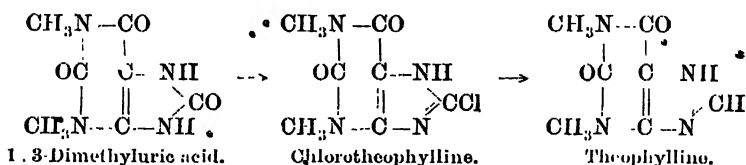
**Synthesis of the Xanthine Bases.** Having established the constitution of the xanthine bases as reduction products of uric acid and the methyl uric acids, the question arises, how can the syntheses of these different products be effected? Uric acid might be reduced to xanthine and the xanthine methylated, or uric acid might be converted into monomethyluric acid, then reduced to monomethyl xanthine and further methylated; or finally, the di- and tri-methyluric acids might be first prepared, and then reduced to the corresponding di- and tri-methyl xanthines. All three methods have been

utilized in turn by Fischer and carried to a successful issue; and since the process is similar in each case, one or two examples may suffice by way of illustration.

When 1.3.7-trimethyluric acid is heated with a mixture of pentachloride and oxychloride of phosphorus, it yields chlorocaffeine. Tetramethyluric acid yields the same product with the elimination of a methyl group in the form of methyl chloride. Chlorocaffeine is then reduced with strong hydriodic acid to caffeine.<sup>1</sup>



1.3-Dimethyluric acid behaves similarly and forms theophylline.



This process cannot, however, be applied to uric acid in order to obtain xanthine or to 3- or 7-monomethyl- or to 3.7-dimethyluric acid, which might lead to the synthesis of theobromine; because in the first case the substance is destroyed and in the other cases the chlorine atom replaces the wrong oxygen atom, i. e. instead of replacing it in position 8, which is essential to the success of the operation, it enters position 6.

The happy idea of employing phosphorus oxychloride alone, in place of the mixture of pentachloride and oxychloride, has overcome this unforeseen difficulty and given a fortunate turn to the investigation.

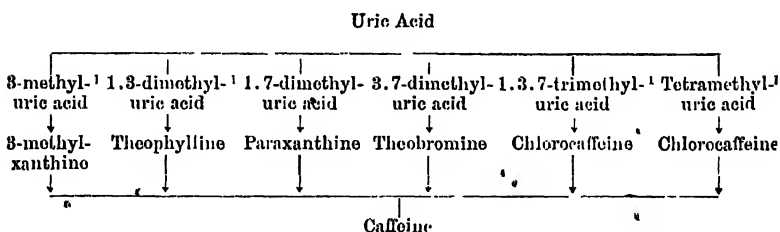
By this modification 3.7-dimethyluric acid may be made to yield chlorotheobromine; or better still, 3-methyluric acid, which can be obtained by the direct methylation of uric acid, may be converted into 3-methyl-8-chloroxanthine. The latter can either be methylated

<sup>1</sup> The direct reduction of the methyluric acids to xanthine bases has never been effected by chemical reagents. Electrolytic methods, which have been investigated by Tafel, have shown that it is the oxygen of the carbonyl group in position 6 which is replaced by hydrogen. *Ber.*, 1901, 34, 279.



with methyl iodide in presence of caustic potash so as to give chlorotheobromine and; by methylation, chlorocaffeine, and then reduced. Or first reduced to 3-methylxanthine and then methylated. As a rule, however, the methylation of the chlorine compound is more easily effected than that of the reduced product. Paraxanthine (1.7-dimethylxanthine) may be obtained from 1.7-dimethyluric acid, and, in a similar manner, converted by methylation into caffeine.

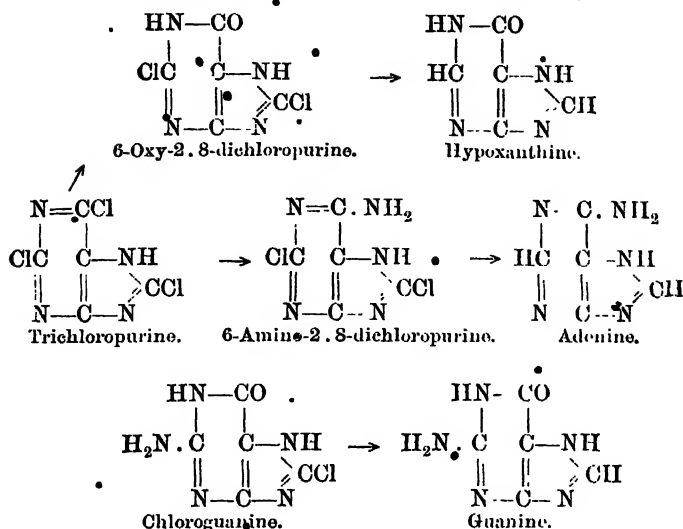
The following scheme will indicate the various directions in which the synthesis of caffeine has been accomplished.



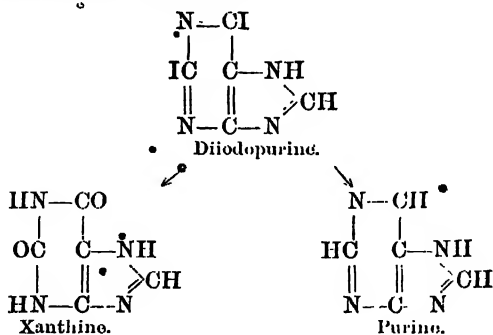
Heteroxanthine (7-methylxanthine) has been obtained by the action of phosphorus oxychloride on theobromine, which, by the elimination of one methyl group, forms 7-methyldichloropurine. By boiling this substance with hydrochloric acid, 7-methylxanthine is formed. Xanthine cannot be prepared from uric acid in so direct and simple a manner as the above, even when phosphorus oxychloride alone is used, for the first product, obtained in this way, is 8-oxy-2.6-dichloropurine; but by the action of a large excess of phosphorus oxychloride, uric acid may be made to part with its last atom of oxygen. Trichloropurine is then produced, and this compound has served for the synthesis of xanthine, its nearly related derivatives, hypoxanthine, adenine, and guanine, and finally, purine, the parent substance of the whole group.

When trichloropurine is treated with aqueous potash it yields 6-oxy-2.8-dichloropurine. The latter compound may be directly reduced with hydriodic acid to hypoxanthine, or converted with alcoholic ammonia into chloroguanine, which on reduction forms guanine. Aqueous ammonia converts trichloropurine into 6-amino-2.8-dichloropurine, which yields adenine on reduction.

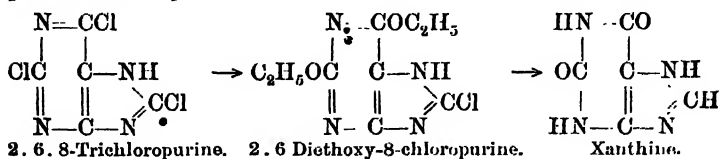
\* These alkyluric acids are formed by the direct methylation of uric acid.



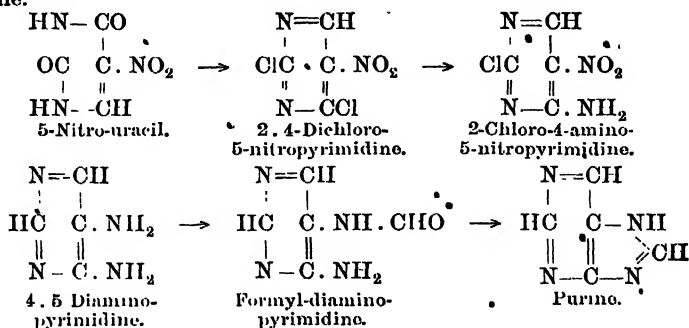
With strong hydriodic acid, trichloropurine is further converted into diiodopurine, which yields, with hydrochloric acid, xanthine, and with zinc dust and water, purine.



An alternative method for the synthesis of xanthine from 2:6:8-trichloropurine is to convert that substance by the action of sodium ethoxide into 2, 6-diethoxy-8-chloropurine, and then to reduce the product with hydriodic acid.

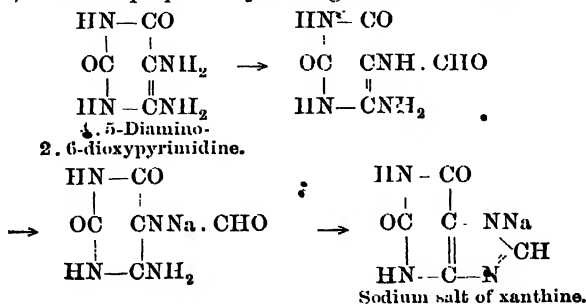


The direct synthesis of purine has recently been carried out by Isay.<sup>1</sup> The starting-point is 5-nitro-uracil, the preparation of which has already been described in connection with the synthesis of uric acid by Behrend and Roosen (p. 110). 5-Nitro-uracil, on being heated under pressure with phosphorus oxychloride, is converted into 2,4-dichloronitropyrimidine, which, on treatment with ammonia, loses one chlorine atom and passes into 2-chloro-4-amino-5-nitropyrimidine. On reduction with hydriodic acid, 4,5-diaminopyrimidine is produced, which is then converted into the formyl derivative. The latter substance, when heated to 210°, loses the elements of water and yields purine.



Purine, although neutral to litmus, forms well characterized salts, and shows surprising stability towards oxidising agents.

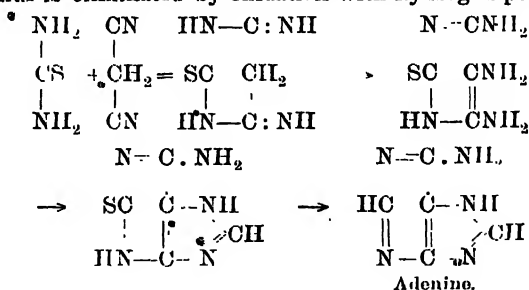
*Traube's Synthesis of Xanthine Bases.* This method closely resembles Traube's synthesis of uric acid (p. 113). Cyanacetylurea is converted, as before, into 4-amino-2,6-dioxypyrimidine: this substance is acted upon with nitrous acid, and the resulting isonitroso compound reduced to 4,5-diamino-2,6-dioxypyrimidine. Xanthine is obtained from the latter substance by heating the sodium salt of its formyl derivative, which is prepared by boiling with formic acid.



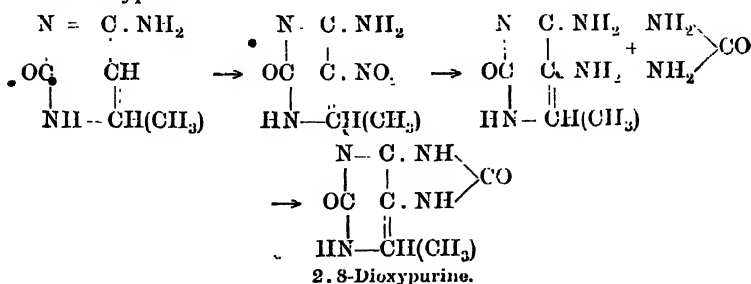
The method has been very widely applied to the synthesis of many

<sup>1</sup> Ber., 1906, 39, 250

other members of the purine group. Thus, if cyanacetylguanidine be substituted for cyanacetylurea, the final product will be guanine, whilst with mono- and dimethyl-cyanacetyl ureas, prepared by condensation of monomethyl- and dimethyl-urea with cyanacetic ester, the products will be 3-methylxanthine and 1,3-dimethylxanthine respectively. Since 3-methylxanthine, on methylation, yields both theobromine and caffeine, it is possible to employ this method for the commercial preparation of these substances. A further extension of the method<sup>1</sup> has led to the synthesis of hypoxanthine and adenine. If thiourea is used as the starting material, and submitted to a similar series of changes to those described above, 2-thio-6-oxypurine will result. On oxidising the latter with dilute nitric acid, hypoxanthine is formed by the elimination of an atom of sulphur. Adenine is obtained by a slightly different series of changes. Thiourea undergoes condensation with methylene cyanide, forming a thio-pyrimidine derivative, which is then converted into a purine compound by the methods employed in the previous syntheses. In the final reaction the sulphur is eliminated by oxidation with hydrogen peroxide.



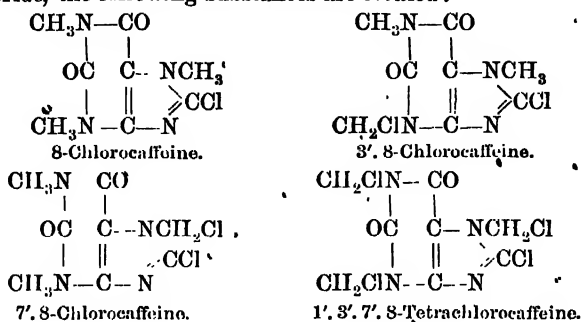
**Johns' Synthesis of Purine Bases.**<sup>2</sup> 6-Amino-uracil and methyl-uracil, prepared by the method already described (p. 109), are nitrated and then reduced to the 5,6-diamino-uracils. On heating with urea the diamino-uracils yield respectively 2,8-dioxy- and 4-methyl 2,8-dioxypurine.



<sup>1</sup> *Annalen*, 1904, 331, 61

*Amer. Chem. J.*, 1909, 41, 58; 1911, 45, 79.

It is of interest to note that whilst caffeine can be prepared by the methylation of various xanthine bases, it is possible to effect the reverse change and to obtain many of the xanthine bases from caffeine by a process of *de*-methylation.<sup>1</sup> By the regulated action of phosphorus pentachloride or of chlorine dissolved in phosphorus oxychloride, the following substances are formed:



It will be seen that chlorine first replaces the hydrogen in position 8, but, on further chlorination, the halogen enters the methyl groups. The chloromethyl groups are easily removed by heating with water, with the liberation of formaldehyde and hydrochloric acid. By this means the above compounds are converted into 8-chloroparaxanthine, 8-chlorotheophylline, and 8-chloroxanthine, which may be reduced to paraxanthine, theophylline, and xanthine respectively.

Heteroxanthine has also been prepared by a similar series of changes.

**The Formation of Uric Acid in the Body.** The relative amount of uric acid excreted varies enormously in different animal species. It forms the greater part of the nitrogenous constituents of the excreta of birds and reptiles, whilst in most mammals, including man, the proportion is only about 2 per cent. The origin of the uric acid in the two cases is entirely different. In the case of birds, ammonia or urea is converted into uric acid by the liver, and there is reason to believe that ammonium lactate is an intermediate product in the reaction. No such conversion of urea into uric acid occurs with mammals, but, on the contrary, the reverse change takes place, and uric acid, taken into the body, is mainly converted into urea. This power of destroying uric acid is generally ascribed to an enzyme in the liver—the so-called *uricolytic enzyme*. For reasons which are unknown this destruction of uric acid is never complete, so that a certain proportion of the uric acid present

<sup>1</sup> Fischer and Ach, *Ber.*, 1906, 39, 423.

at any time in the body escapes by way of the urine. It is found that the uric acid present in the urine of mammals is derived from three sources. The use of certain substances as food, such as thymus, pancreas, and liver, which are rich in nucleo-proteins, is found to give rise to an increased uric acid excretion. The nucleo-proteins contain one or more of the purine bases, xanthine, guanine, hypoxanthine or adenine in combination, and these bases are converted into uric acid by the joint agency of the enzymes adenase, guanase, and xanthine oxidase (see pp. 77, 81). Only a portion of the uric acid so formed is eventually found in the urine. The term 'exogenous uric acid' has been employed to distinguish the uric acid directly derived from food from that produced in other ways. Since uric acid is excreted during prolonged starvation, or when the diet is free from purine bases, it is necessary to assume that at least part of the uric acid normally excreted is derived from the metabolism of the tissues and is independent of the food-supply. The term 'endogenous uric acid' is applied to uric acid which originates in this way, and may arise from the nucleo-proteins of disintegrated cells. Since nucleo-proteins taken in as food yield some part of their purine bases in the form of uric acid, it is natural to suppose that the breaking down of the nucleo-proteins of degenerating body cells would also yield uric acid. That this is probably the case is inferred from the fact that conditions which cause a large increase in the production (and destruction) of nucleated leucocytes (such as follows the use of certain drugs and is seen in leucaemia and other diseases), result in an increased uric acid excretion.

A third source of uric acid is found in *purine bases produced in metabolism*. The important fact has recently been recognized that purine bases may be produced in normal cell metabolism. It has been shown that hypoxanthine is found in the muscles, and that the quantity is increased during activity. The hypoxanthine does not leave the muscle as such, but is converted into uric acid by the action of an oxidising enzyme. The mode of production of the hypoxanthine is unknown. It is certain that the synthesis of purine bases is readily carried out in the living cell, because nucleo-proteins containing purine bases are formed in the development of young animals, and the latter derive their nourishment from food which need contain no purine derivatives.

#### REFERENCES.

- Synthesen in der Puringruppe*, by E. Fischer. *Ber.*, 1899, 32, 436  
*Vegetable Alkaloids*, by A. Pictet, trans. by H. C. Biddle. Wiley, New York, 1904.

## CHAPTER IV

### THE PROTEINS

THE proteins embrace a large and ill-defined group of substances which are derived directly or indirectly from living matter. They form the chief constituents of the protoplasm of the majority of cells and enter into the composition of animal tissues and secretions. A knowledge of their structure is clearly of the greatest importance to biological science. But their study offers peculiar difficulties. They are colloidal, non-volatile substances, and consequently difficult to obtain in a state of purity. Moreover, they do not lend themselves to investigation by simple chemical methods. The early investigators who turned their attention to the better known but more complex proteins, apart from collecting a number of interesting empirical facts, obtained little insight into their real nature.

At a later period the simpler members of the group, such as the protamines, were examined with more success by Kossel and others. Still more recently the comprehensive study of the simplest constituents or constitutive fragments of the protein molecule which was carried out by Emil Fischer and his co-workers, has thrown a flood of light on the subject. These constituents consist mainly of amino acids, for which new methods of separation, identification, and synthesis have been devised, so that the structure of the majority of them is now well known. The knowledge gained by these investigations has clearly indicated the lines upon which the construction of the simpler members of the protein group may be accomplished, and although we are still ignorant of the constitution of even the simplest protein, the problem has lost something of the hopeless aspect that it formerly presented.

Before considering individual members of the protein group, it will be convenient to give a brief outline of the methods employed in their investigation. Animal and vegetable tissues and fluids must of necessity serve as the material for their preparation. The plan almost universally adopted consists in *precipitating* by various means the proteins contained in the aqueous extracts, prepared by digesting the tissues with dilute alkali, acid, or salt solutions.

A great number of substances have been employed as precipitants, of which the most important are ammonium sulphate, sodium chloride, sodium sulphate, zinc sulphate, magnesium sulphate or organic liquids, such as alcohol, ether, and acetone. Frequently a protein may be precipitated from an alkaline solution by acidifying with some weak acid like acetic or carbonic acid. All proteins are precipitated on complete saturation of the solution with ammonium sulphate, a reagent which usually produces less alteration in unstable substances than alcohol or other similar precipitants.

The various proteins differ widely in the relative ease with which they are precipitated by salts, and it has been found that systematic 'salting out' forms one of the best available means of purification. It has long been known that *oxyhaemoglobin* (p. 167) and certain of the vegetable proteins may be obtained in the form of well-defined crystals, and, more recently, other proteins such as egg- and serum-albumin have been obtained in crystalline form by salting out under special conditions. Even when crystalline the proteins may contain many impurities arising partly from their power of adsorption, nevertheless, by repeated precipitation or crystallization, a number have been isolated which are believed to be individual substances of definite composition.

The proteins contain carbon, hydrogen, nitrogen, and oxygen, and most of them sulphur and phosphorus in addition. The relative proportion of carbon, hydrogen, and nitrogen in the majority of typical proteins varies within small limits, as will be seen from the following average numbers: carbon, 52-55 per cent.; nitrogen, 15-19 per cent.; hydrogen, 6.5-7.5 per cent. The molecular weights of the proteins are extremely high, but in every case exact determinations are still wanting. From a consideration of the products of hydrolysis, it is concluded that the simple protamines have a molecular weight of some multiple of 2,000, while the assumption that the complicated protein, *haemoglobin* (approximately  $C_{718}H_{1203}O_{135}N_{21}FeS_3$ ), contains only one atom of iron in the molecule, leads to a minimum molecular weight of about 16,600.<sup>1</sup> Similar numbers are obtained from calculations based upon the proportions in which carbonic oxide or oxygen combine with haemoglobin.

The employment of physical methods of molecular weight determination has not led to completely satisfactory results, but they at least confirm the enormously high estimates made on chemical grounds. By the use of the freezing-point method, a molecular

<sup>1</sup> *Zeit. physiol. Chem.*, 1889, 14, 289.



weight of about 14,000 has been assigned to egg-albumin. Careful direct measurements of osmotic pressure in solutions of certain proteins have been made by Waymouth Reid<sup>1</sup>; but, with the exception of haemoglobin, negative results were uniformly obtained. Assuming the molecular weight of haemoglobin to be 16,600, as deduced from its iron-content, it is calculated that, if no dissociation in solution occurred, a 1 per cent. solution of the protein would give a pressure of 10.7 mm. of mercury at 15°. Since the observed pressures are only about one-third of this, the true molecular weight appears to be a multiple of the minimal number calculated from analysis. In this connection it is of interest to note that the appearance of solutions of haemoglobin, when examined in a special form of microscope of high power, resembles that of distilled water, and presents a marked contrast to the milky appearance of the pseudo-solutions of certain other proteins. It is therefore probable that haemoglobin, unlike most other proteins, forms a true solution in water.

Taking advantage of the fact that certain proteins, such as casein, act as polybasic acids and form neutral salts with alkalis which are ionised in solution, Sackur<sup>2</sup> has deduced molecular weights from electrical conductivity measurements. In the case of casein, the combining proportions of sodium hydrate and protein lead to an equivalent of 1,135 for the latter, whilst the conductivity measurements indicate that this number must be multiplied from four to six times to give the molecular weight.

The wide variations in the molecular weight, estimated by different methods, renders it impossible to place much reliance upon the numbers, but as the experimental errors in most cases tend to give low results, it is probably safe to assume that few proteins have a molecular weight of less than 10,000.

One of the most characteristic properties of the proteins is the curious transformation which most of them exhibit when their solutions are heated. At a certain definite temperature the protein undergoes coagulation, forming an insoluble clot, which does not redissolve on cooling. The exact temperature at which coagulation takes place varies with different proteins and is influenced by the reaction of the solution and by the presence or absence of salts. The clotted proteins are much more insoluble and generally less reactive substances than the parent proteins, and they cannot be reconverted into the original coagulable proteins.

<sup>1</sup> *Journ. of Physiol.*, 1904, 31, 438; 1905, 33, 12.

<sup>2</sup> *Hofmeisters Beiträge*, 1902, 3, 191.

The proteins can function both as acids and as bases. Some, such as casein, have pronounced acid properties, whilst others, such as the protamines, are strongly basic substances, but indications of both basic and acid nature may be found in all. The explanation of this phenomenon may be referred to the presence in the protein molecule of amino-acid groups, in which there is a balance between the basic-amino and acid-carboxyl groups. If a portion is warmed with dilute acid it is converted into 'acid-albumin', while on similar treatment with caustic alkali it is rapidly changed to an 'alkali albuminate'. The nature of the reactions taking place is still unknown, but the products of the action of acids and bases upon proteins show great variations in properties from the parent substances, and their aqueous solutions no longer undergo typical coagulation on heating.

**Colour Reactions of the Proteins.** A number of colour reactions have been described which were at one time believed to be characteristic of the proteins.\* It has, however, been found that most of these reactions are due to the presence of special amino-acid groups in the protein molecule, to which reference is made later.

*The Biuret Reaction.* A fine pink or violet colour is produced on the addition of an excess of caustic soda and a trace of copper sulphate to a solution containing proteins. This reaction is given by all proteins, as well as by proteoses, peptones (p. 151), and all except a few of the simplest synthetical polypeptides (p. 151), but the test fails with the free amino acids. The tint varies considerably, but is usually bluish-violet with proteins and pink with the peptones. In addition to the protein derivatives, already mentioned, certain other substances, including biuret, malonamide, and oxamide, give the reaction. Schiff<sup>1</sup> has attempted to correlate those substances which yield the biuret reaction with their structure, but his results are no longer accepted as entirely trustworthy. It is probable that the reaction in the case of the proteins is due to groups which result from the condensation of amino-acid molecules among themselves, and as the dipeptides and many tripeptides do not give a distinct reaction, several of these groups appear to be necessary.

*The Xanthoproteic Reaction.* On addition of concentrated nitric acid to a protein solution, followed by gentle warming, a deep yellow colour results which changes to orange on the addition of ammonia. The reaction is due to the production of coloured aromatic nitro compounds. It is given by nearly all proteins; for, with the

<sup>1</sup> *Ber.*, 1896, 20, 298; *Annalen*, 1897, 299, 236; 1901, 310, 300.

exception of the protamines, they all, contain heterocyclic phenylalanine or tyrosine groups (p. 148).

*Millon's Reaction.* Almost all proteins, with the exception of gelatine and the protamines, contain tyrosine groups in their molecule, and hence give a red colour on warming with Millon's reagent.

*Adamkiewicz-Hopkins Reaction.* A fine bluish-violet colour is produced on adding half a volume of concentrated sulphuric acid to a protein solution containing a trace of glyoxalic acid.<sup>1</sup> The production of the colour depends upon the presence of tryptophane groups (p. 147), and a positive reaction is therefore obtained with all proteins with the exception of gelatine and the protamines.<sup>2</sup>

*Lead Sulphide Reaction.* The majority of proteins contain sulphur in the form of cystine groups (p. 143) and give a black precipitate of lead sulphide on boiling with caustic soda and lead acetate solution.

*Molisch Reaction.* A violet colour is produced on adding strong sulphuric acid to a protein solution containing an alcoholic solution of  $\alpha$ -naphthol. This reaction is given by all proteins which contain carbohydrate complexes and depends upon the production of furfural by the action of the mineral acid.

On account of their physical properties the proteins do not lend themselves readily to direct investigation. Their structure and composition, as far as they have been elucidated, have been arrived at almost exclusively from a study of their products of hydrolysis and of oxidation. Of these two methods hydrolysis has proved to be much the most fruitful, in fact, the modern chemistry of the proteins is mainly concerned with the products obtained by various methods of hydrolysis. The methods are of two kinds, either purely chemical, in which mineral acids, alkali, or superheated steam are employed, or biochemical, in which the proteins are resolved into simpler products by means of enzymes which normally occur in living organisms, particularly in the digestive tract of animals. The hydrolysis of the proteins by enzymes is effected at low temperatures and in the absence of more than traces of acid or alkali. As the reaction occurs in several fairly well-defined stages, it has been possible to obtain from the complex proteins a number of products, intermediate in character between the simple end-products of complete hydrolysis and the parent substance. The importance of this method can hardly be over-estimated, especially

<sup>1</sup> Ordinary glacial acetic acid usually contains traces of glyoxalic acid and was originally employed for this test in place of the pure acid.

<sup>2</sup> A protamine named cycloptertine, however, contains tryptophane groups, and consequently gives a positive reaction.

as the course of the reaction may be varied to some extent by the employment of different enzymes. The more important products of protein hydrolysis, arranged in order of their complexity, are proteoses, peptones (including simple polypeptides), and amino acids. The first two classes of compounds will be referred to at a later stage, after the simpler amino acids have been discussed.

**The Amino Acids.** If a protein, for example egg-albumin or casein, be completely hydrolysed by boiling with concentrated hydrochloric acid, a clear, dark-coloured solution is obtained which no longer gives the biuret reaction. The problem of separating and identifying the products has long taxed the chemists' ingenuity. As long ago as 1820 Braconnot obtained glycine and leucine from gelatine, and thirty years later Liebig found tyrosine among the decomposition products of horn. Leucine and tyrosine were then so frequently encountered that these two amino acids were thought to comprise the bulk of the protein decomposition products. With the employment of better methods, other amino acids, such as aspartic and glutamic acids, were added to the list, and a great advance was made in 1889 when Drechsel showed that a considerable proportion of the products of protein hydrolysis were strongly basic substances belonging to the class of diamino acids. Ten years later Mörner was able to demonstrate the wide distribution of the sulphur-containing amino acid, cystine, whilst an acid of an entirely new type—tryptophane, a derivative of indole—was isolated by Hopkins and Cole. The recent introduction by Fischer of improved methods for the separation of amino acids, based upon their conversion into volatile esters (which can be partially separated by fractional distillation *in vacuo*), has led to the recognition of the wide distribution of acids such as alanine, serine, and phenylalanine, which had only been previously detected in the products from a few proteins, and to the discovery of two cyclic acids of a new type,  $\alpha$ -pyrrolidinecarboxylic acid and hydroxy-pyrrolidinecarboxylic acid.

The following interesting table is taken from Dr. Plimmer's monograph on 'The Chemical Constitution of the Proteins.'<sup>1</sup>:

Substance.	Discovered		Racemic 'dl' Form synthesised		Natural Active Form obtained	
	by	in	by	in	by	in
Glycine	Braconnot	1820	Perkin and Duppa	1858	—	—
Alanine	Schützenberger and Weyl	1888	Strecker	1850	Fischer	1899

<sup>1</sup> *Monographs on Biochemistry*, Longmans 1912.

Substance.	Discovered		Racemic 'dl' Form synthesised			Natural Active Form obtained	
	by	in	by	in		by	in
Valine	v. Gorup-Besanez	1856	Fittig and Clark	1866	d	Fischer	1906
Caprin	Abderhalden and Weie	1913	—	—	—	—	—
Leucine	Proust, Braconnot	1820	Limpricht, Schulze and Likiernik	1855 1885	l	Fischer	1900
Isoleucine	Ehrlich	1903	Bouveault & Locquin	1905	d	Locquin	1907
Phenylalanine	Schulze and Barbieri	1881	Erlenmeyer and Lipp	1883	l	Fischer and Schöller	1907
Tyrosine	Liebig	1846	„	1883	l	Fischer	1900
Serine	Cramer	1865	Fischer and Leuchs	1902	l	Fischer and Jacobs	1906
Cystine	{ Wollaston Mörner	1810 1899	Erlenmeyer jun.	1903	l	Fischer and Raske	1908
Aspartic acid	Plisson	1827	Dessaignes	1850	l	Piutti	1887
Glutamic acid	Ritthausen	1866	Wolff	1890	d	Fischer	1899
Ornithine	Jaffé	1877	Fischer	1900	d	Sørensen	1905
Arginine	Schulze and Steiger	1886	Schulze and Winterstein, Sørensen	1899 1910	d	—	—
Lysine	Drechsel	1889	Fischer and Weigert	1902	d	—	—
Proline	Fischer	1901	Willstätter	1900	l	Fischer and Zemplén	1909
Hydroxyproline	„	1902	Leuchs (?)	—	l	—	—
Histidine	Kossel	1896	Pyman	1911	l	Pyman	1911
Tryptophane	Hopkins and Cole	1901	Ellinger and Flamand	1907	l	—	—
$\beta$ -Hydroxy-glutamic Acid	Dakin	1918	Dakin	1919	—	—	—

( **Products of Protein Hydrolysis.** Hydrolysis may be effected by boiling with mineral acids (hydrochloric, sulphuric, or hydrofluoric) or alkalis, or by the action of certain enzymes, such as pepsin and trypsin (p. 77), which possess the property of breaking down the proteins into simpler products; complete hydrolysis is indicated by the biuret reaction. The different kinds of amino-acids present in the products of protein decomposition may be classified as follows :

Monobasic monamino acids

Dibasic monamino acids

Diamino acids

Hydroxy- and thio-monamino acids

Heterocyclic amino acids

Aromatic amino acids.

The following table contains the chief amino acids which have been isolated from the products of protein hydrolysis :

*Monobasic Monamino acids.*

Glycine = aminoacetic acid	$(\text{H}_2\text{N})\text{CH}_2\text{COOH}$
Alanine = $\alpha$ -aminopropionic acid	$\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$
Valine = $\alpha$ -aminoisovaleric acid	$\begin{array}{c} \text{CH}_3 \\   \\ \text{CH} \\   \\ \text{CH}_3 \end{array} \text{CH}(\text{NH}_2)\text{COOH}$
Leucine = $\alpha$ -aminoisobutylic acid	$\begin{array}{c} \text{CH}_3 \\   \\ \text{CH} \\   \\ \text{CH}_3 \end{array} \text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$
Isoleucine = secondary butyl $\alpha$ -aminoacetic acid	$\begin{array}{c} \text{C}_2\text{H}_5 \\   \\ \text{CH} \\   \\ \text{CH}_3 \end{array} \text{CHCH}(\text{NH}_2)\text{COOH}$
Caprine = $\alpha$ -amino caproic acid.	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$

*Dibasic Monamino acids.*

Aspartic acid = aminosuccinic acid	$\begin{array}{c} \text{CH}(\text{NH}_2)\text{COOH} \\   \\ \text{CH}_2\text{COOH} \end{array}$
Glutamic acid = aminoglutaric acid	$\begin{array}{c} \text{CH}(\text{NH}_2)\text{COOH} \\   \\ \text{CH}_2\text{CH}_2\text{COOH} \end{array}$

*Hydroxy- and Thio-amino acids.*

Serine = $\alpha$ -amino - $\beta$ -hydroxypropionic acid	$\text{CH}_2(\text{OH})\text{CH}(\text{NH}_2)\text{COOH}$
Hydroxyglutamic acid	$\text{COOH} \cdot \text{CH}_2 \cdot \text{CH}(\text{OH}) \cdot \text{CH}(\text{NH}_2)\text{COOH}$
Trihydroxydiaminododecaic acid	$\text{C}_{11}\text{H}_{16}(\text{OH})_3(\text{NH}_2)_2\text{COOH}$
Cysteine = $\alpha$ -amino- $\beta$ -thiolactic acid	$\text{CH}_2(\text{SH})\text{CH}(\text{NH}_2)\text{COOH}$
Cystine	$\begin{array}{c} \text{S} \cdot \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \\   \\ \text{S} \cdot \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \end{array}$

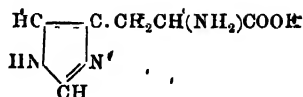
*Diamino acids.*

Ornithine = $\alpha$ - $\delta$ -diaminovaleric acid	$(\text{H}_2\text{N})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
Lysine = $\alpha$ - $\epsilon$ -diaminocaproic acid	$(\text{H}_2\text{N})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
Arginine = $\alpha$ -amino - $\delta$ -guanidovaleric acid	$\begin{array}{c} \text{NH} \\    \\ (\text{H}_2\text{N})\text{C} - \text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \end{array}$

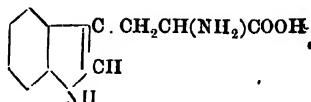
✓ *Heterocyclic Amino acids.*

Proline = $\alpha$ -pyrrolidine carboxylic acid	$\begin{array}{c} \text{H}_2\text{C} \quad \text{CH}_2 \\   \quad \diagup \\ \text{H}_2\text{C} \quad \text{CH} \cdot \text{COOH} \\   \\ \text{NH} \end{array}$
Oxyproline = hydroxypyrrolidine carboxylic acid	$\begin{array}{c} \text{HO HC} - \text{CH}_2 \\   \\ \text{H}_2\text{C} \quad \text{CH} \cdot \text{COOH} \\   \\ \text{NH} \end{array}$

Histidine =  $\alpha$ -amino -  $\beta$ -iminazole-  
propionic acid

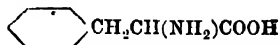


Tryptophane = indole-aminopropi-  
onic acid

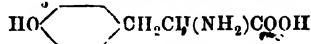


*Aromatic Amino acids.*

Phenylalanine =  $\alpha$ -amino- $\beta$ -phenyl-  
propionic acid



Tyrosine =  $\alpha$ -amino - *p*-hydroxy-  
phenyl-propionic acid

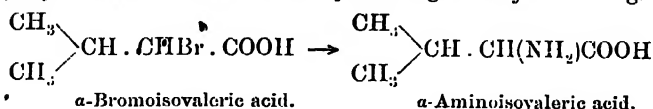


It will be seen from the table that there are about twenty different amino acids, the majority of which are commonly found among the products of hydrolysis of typical proteins, although their relative proportions vary considerably. Casein, for example, yields all the above-mentioned products of hydrolysis with the possible exception of glycocoll. These acids are all  $\alpha$ -amino acids, and constitute practically the whole of the hydrolytic products of the proteins; a variable quantity of ammonia (0.4-5.0 per cent.) is usually set free, and, in the case of hydrolysis by acids, certain secondary decomposition products are commonly met with.<sup>1</sup> The mode in which the different amino acids are united in the protein molecule will be considered at a later stage.

*Monobasic Monamino Acids.* The monobasic monamino acids form a large part of the products of hydrolysis of most proteins. That they serve as a source of energy available for the animal organism naturally follows from the fact that they are produced in large quantities in the process of intestinal digestion of protein food. They are all crystalline, sweet-tasting substances which are soluble in water, but insoluble in alcohol and ether. They have a neutral reaction, but form well-defined crystalline salts with both acids and bases. With the exception of glycine they all contain asymmetric carbon atoms, and they are known in both racemic and active forms, one of the latter being present in protein decomposition products. All the amino acids of this group have long been known, and their synthesis has been accomplished by means of the five following reactions:

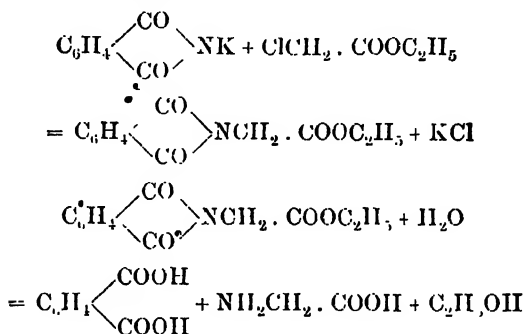
<sup>1</sup> Details of the methods of separation and identification of the amino acids will be found in Plimmer's *Chemical Constitution of the Proteins*; Monographs on Biochemistry, Longmans, 1912.

(a) By the action of ammonia upon halogen fatty acids, e. g.



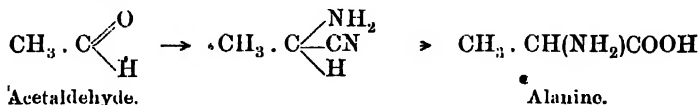
The  $\alpha$ -bromo fatty acids, necessary for the synthesis, may be prepared by the ordinary methods of direct bromination, but in many cases better yields are obtained by brominating the corresponding alkylmalonic acid and then converting the product into a monobasic acid by distillation.<sup>1</sup>

(b) By Gabriel's method,<sup>2</sup> which consists in combining potassium phthalimide with halogen acid ester and hydrolysing the product. Glycine is prepared as follows :



In place of the halogen fatty acid the monohalogen malonic ester may be used, whereby it is possible to introduce into the malonic ester group an additional alkyl radical. The product is then hydrolysed, and carbon dioxide removed from the amino malonic ester (p. 140).

(c) By Strecker's method, which consists in combining an aldehyde with ammonia and hydrocyanic acid and hydrolysing the resulting amino-cyanhydrin, e. g.



(d) By the method of Erlenmeyer, jun.,<sup>3</sup> who condenses aldehydes and esters with hippuric acid in presence of acetic anhydride, then

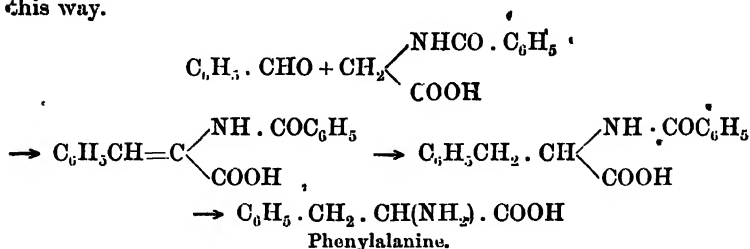
<sup>1</sup> E. Fischer, *Ber.*, 1901, 37, 3062 ; 1906, 39, 351.

<sup>2</sup> Gabriel and Kroseberg, *Ber.*, 1889, 22, 426.

<sup>3</sup> *Annalen*, 1893, 275, 1; 1899, 307, 70, 163.



reduces and hydrolyses the product. Phenylalanine was prepared in this way.



(e) Another method, which has been occasionally used by Bouveault and Locquin,<sup>1</sup> is to convert the ketonic acid into the oximino-derivative and reduce the oximino- to the amino-group.

The optically active acids are obtained from the racemic forms, in some cases by the actions of organisms; but more generally by a method which is due to E. Fischer.<sup>1</sup> It consists in the conversion of the amino acids into strongly acid benzoyl- or formyl derivatives, which yield well-crystallized salts with alkaloids, capable of separation by fractional crystallization (Part II, p. 183). The optically active acyl derivatives yield the active amino acids on hydrolysis. *d*-Camphor-sulphonic acid and its bromine derivative (Part II, p. 183) have also been used for resolving the amino esters.<sup>2</sup> Ehrlich<sup>3</sup> has obtained active amino acids by fermentation with yeast, one of the enantiomorphs being assimilated in the process (Part II, p. 181).

**Dibasic Monamino Acids.** Two dibasic monamino acids, aspartic and glutamic acids, are important constituents of many protein decomposition products. In the case of the proteins from wheat, the yield of glutamic acid may exceed 30 per cent. Aspartic and glutamic acids, as might be anticipated, are strongly acid substances which form well-defined metallic salts, but they still retain the power of combining with acids.

Inactive aspartic acid has been synthesised by heating fumaric acid with ammonia,<sup>4</sup> and the *d*-acid, by acting upon *l*-bromo succinic acid with ammonia.<sup>5</sup>

Piutti<sup>6</sup> also obtained the inactive acid by the action of hydroxylamine on oxalacetic ester and then reducing the isonitroso-succinic ester compound:

<sup>1</sup> Ber., 1899, 32, 2451.

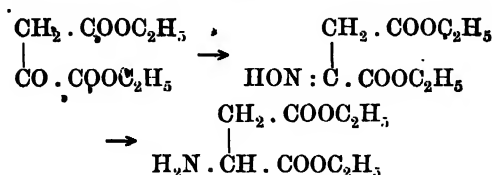
<sup>2</sup> Ber., 1908, 41, 2071; Gazz. chem. ital., 1914, 44, 97.

<sup>3</sup> Ber., 1907, 40, 1028.

<sup>4</sup> Engel, Compt. rend., 1887, 104, 1805.

<sup>5</sup> Fischer and Raske, Ber., 1907, 40, 1051.

<sup>6</sup> Gazz. chem. ital., 1887, 17, 519.



Glutamic acid was obtained by the reduction of  $\alpha$ -isonitroso-glutaric acid.<sup>1</sup>

*The Diamino Acids.* The diamino acids are strongly basic substances, which are among the most constant and characteristic products of protein hydrolysis. The first members were discovered by Drechsel, who isolated lysine and a substance which he named *lysatinine*. The latter was subsequently shown by Hedin to be a mixture of lysine with a base named *arginine*, which had previously been detected in lupine seedlings by E. Sclfulze. Somewhat later a third base, *histidine*, was independently discovered by Kossel and by Hedin, and for some time it was thought that this substance might be classified with lysine and arginine, but recent work has shown that it has an entirely different constitution (see p. 145). These three bases, lysine, arginine, and histidine, each contain six carbon atoms and are frequently spoken of as the *hexone* bases, but since the constitution of these substances has been ascertained the term seems scarcely suitable.

Arginine is perhaps the most widely distributed of the amino acids, and although the amount may be small, there is no protein which does not yield this substance on hydrolysis; thus *elastin*, a connective tissue protein, yields only 0.3 per cent., while as much as 90 per cent. is found in many protamines. It is completely resistant to the action of acids, but is easily decomposed by alkali or by an enzyme *arginase* yielding urea and a new diamino acid, *ornithine* (p. 79).

From the formulae of these substances it will be seen that they all contain an amino group in the  $\alpha$ -position and a straight chain of five or six carbon atoms.

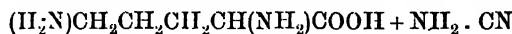
- Ornithine =  $(\text{NH}_2)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
- Lysine =  $(\text{NH}_2)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
- Arginine =  $(\text{NH}_2 \cdot \text{C} : \text{NH} \cdot \text{NH})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$

A clue to the constitution of ornithine and lysine was furnished by the observation of Ellinger,<sup>2</sup> who found that these substances are

<sup>1</sup> Wolff, *Annalen*, 1890, 260, 79.

<sup>2</sup> *Zeit. physiol. Chem.*, 1900, 20, 334.

converted by the action of putrefactive organisms into tetramethylenediamine and pentamethylenediamine respectively, whilst the relation of arginine to ornithine was made clear by Schulz's<sup>1</sup> synthesis of the former substance by the direct addition of cyanamide to ornithine.

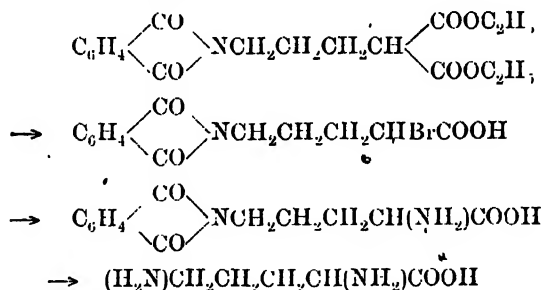


Ornithine.



That the guanidine group is attached to the end of the chain, and not to the  $\alpha$ -carbon, has been shown by Sørensen.<sup>2</sup>

The synthesis of ornithine and lysine offered considerable difficulties, but these have been successfully overcome by E. Fischer<sup>3</sup> and later by Sørensen.<sup>4</sup> Phthalimidopropylmalonic ester, which served as the starting-point for Fischer's synthesis of ornithine, was converted into phthalimido- $\alpha$ -bromovaleric acid by bromination; the bromine was then replaced by an amino group by means of ammonia, and the product gave inactive ornithine on removal of the phthalyl group by hydrolysis.



Sørensen's synthesis is very similar to the above. A more convenient method was afterwards introduced by Fischer and Zemplén.<sup>5</sup> When benzoyl-piperidine is oxidised with permanganate, cleavage of the ring follows, and the resulting benzoyl  $\delta$ -aminovaleric acid is then converted, by the action of bromine and phosphorus, into the bromo-derivative. From the latter monobenzoyl-ornithine acid is obtained by treatment with ammonia, and from this ornithine by hydrolysis.

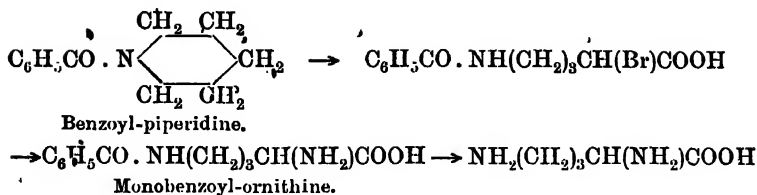
<sup>1</sup> *Ber.*, 1899, 32, 3191.

<sup>3</sup> *Ber.*, 1901, 34, 454; 1902, 35, 3772.

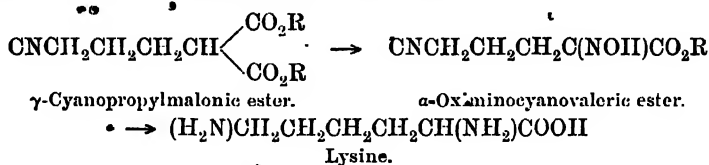
<sup>4</sup> *Compt. rend. trav. Laborat. Carlsberg*, O.

<sup>2</sup> *Ber.*, 1910, 43, 648.

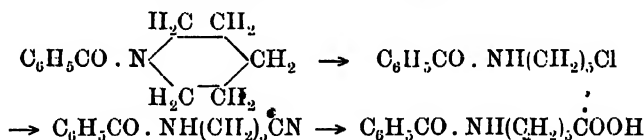
<sup>5</sup> *Ber.*, 1909, 42, 1022.



Fischer's synthesis of lysine depends upon the conversion by nitrous acid of  $\gamma$ -cyanopropylmalonic ester into an oximino derivative of a monobasic acid. This substance on reduction with sodium and alcohol yields  $\alpha$ - $\epsilon$ -diaminocaproic acid (lysine).



Lysine has also been obtained by v. Braun<sup>1</sup> from benzoylpiperidine, which on treatment with phosphorus pentachloride yields the open-chain chlorine compound, which is then converted successively into the nitrile and the acid. The succeeding steps correspond to those of Fischer and Zemplén described above.



Ornithine is not usually found among the products of hydrolysis of proteins by acids. Lysine, on the other hand, is a very general constituent of the hydrolytic products of proteins, and is absent only from certain protamines and from a few vegetable proteins such as zein (p. 164).

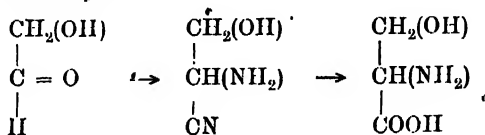
Ornithine, arginine, and lysine, like many other bases, are precipitated from acid solutions by means of phosphotungstic acid. The other amino-acids, with the exception of some of the heterocyclic acids (histidine and  $\alpha$ -pyrrolidine-carboxylic acid), are not precipitated by this reagent, which is therefore used as a means of separation.<sup>2</sup>

*Hydroxy- and Thio-monamino Acids.* The presence of serine ( $\alpha$ -amino- $\beta$ -hydroxypropionic acid) among the products of hydrolysis of the

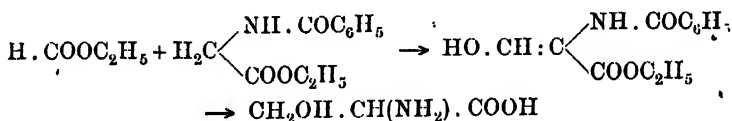
<sup>1</sup> Ber., 1909, 42, 839.

<sup>2</sup> Methods for the approximate quantitative determination of lysine, arginine, and histidine, are given by Kassel and Kutscher, Zeit. physiol. Chem., 1900, 31, 165.

proteins found in saw silk was observed as long ago as 1865 by Cramer,<sup>1</sup> but it is only within recent years that it has been recognized as a common protein constituent. Its isolation offers considerable difficulties, which are in part avoided by the employment of Fischer's ester method of separation (p. 150). Serine contains an asymmetric carbon atom, and proteins yield the laevo form. Its constitution follows from its conversion into glyceric acid by the action of nitrous acid, from its reduction to alanine by hydriodic acid, and from its synthesis which has been accomplished by Fischer and Leuchs,<sup>2</sup> and also by Erlennmeyer.<sup>3</sup> The two former obtained a small quantity of serine by the hydrolysis of the aminocyanhydrin derived from glycollic aldehyde.

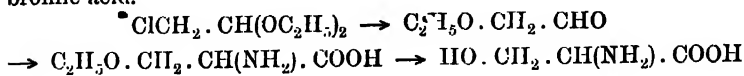


Erlennmeyer condensed formic ester with hippuric ester in presence of sodium ethoxide, and the resulting product was reduced with the aluminium-mercury couple.



Leuchs and Geiger<sup>4</sup> also obtained it from chloroacetal, which was converted with sodium ethoxide into ethoxyacetal, then into the aldehyde, and by Strecker's method into the amino acid.

Finally, the ethoxyl group was replaced by hydroxyl with hydrobromic acid.



The inactive compound was subsequently resolved by Fischer and Jacobs<sup>5</sup> by means of the *p*-nitrobenzoyl derivative; the quinine or brucine salt of which was fractionally crystallized.

Although it is likely that hydroxyamino acids constitute an important part of the fragments of the protein molecules, serine,

<sup>1</sup> *J. prakt. Chem.*, 1865, 99, 76.

<sup>2</sup> *Ber.*, 1902, 35, 3787; 1906, 39, 2942; 1907, 40, 1501.

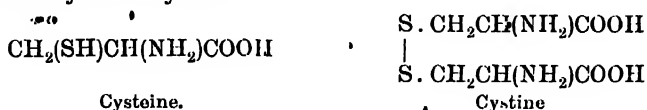
<sup>3</sup> *Ber.*, 1902, 35, 3769.

<sup>4</sup> *Ber.*, 1906, 39, 2644.

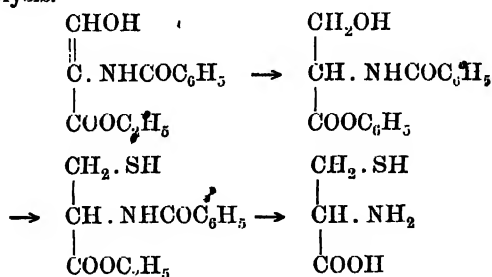
<sup>5</sup> *Ber.*, 1906, 39, 2918.

diamino-trihydroxy-dodecanic acid, and hydroxypyrrolidine carboxylic acid are the only representatives of this class of substances which have so far been isolated.

The larger part of the sulphur of most proteins is found among the products of hydrolysis as cystine or cysteine. The latter substance is the sulphur analogue of serine, whilst cystine is the corresponding disulphide.<sup>1</sup> Apparently cystine is the primary product, but it may be partly reduced to cysteine during hydrolysis. The same reduction is readily brought about by zinc and dilute sulphuric acid, while, on the other hand, cysteine dissolved in dilute ammonia is readily oxidised by air to cystine.



Cysteine, unlike serine, is powerfully laevo-rotatory, although it is partially racemised in the process of hydrolysis. It is readily prepared from horn or hair, which may yield from seven to fourteen per cent.<sup>2</sup> The racemic form has been obtained by Erlenmeyer, jun.,<sup>3</sup> by methods similar to those which he employed in the synthesis of serine. Ethylformyl hippurate, prepared by the condensation of formic and hippuric esters, forms, on reduction, the ester of benzoylserine, and this substance yields on treatment with phosphorus pentasulphide a thio-derivative, from which cysteine may be obtained on hydrolysis.



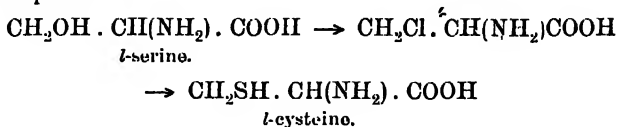
Fischer and Raske also obtained it from *l*-serine by converting it with phosphorus chloride into  $\beta$ -chloro  $\alpha$ -amino propionic acid and

<sup>1</sup> According to Baumann's original formulæ for cysteine and cystine, the nitrogen and sulphur atoms were attached to the same carbon atoms. The present formula is determined by the work of Friedmann, *Beit. chem. Phys. u. Path.*, 1902, 2, 433, and Neuberg, *Ber.*, 1902, 35, 3161.

<sup>2</sup> K. A. H. Möhrner, *Zeit. physiol. Chem.*, 1899, 28, 599; 1901, 34, 207; 1904, 42, 347.

<sup>3</sup> *Annalen*, 1904, 307, 236.

then replacing the halogen by the SH group by means of barium hydrosulphide.<sup>1</sup>



Cysteine, as already mentioned, may be readily oxidised to cystine by passing air through the solution.

Cystine and cysteine are substances of considerable physiological importance, since many other compounds, such as hydrogen sulphide, methyl mercaptan, ethyl mercaptan, ethyl sulphide, taurine, and other substituted sulphuric acids, are obtained by their decomposition through the agency of living organisms.

*Heterocyclic Amino Acids.*  $\alpha$ -Pyrrolidine carboxylic acid (proline) is the simplest member of this group of amino acids. It was discovered by Fischer<sup>2</sup> among the products of hydrolysis of casein and identified with the synthetical acid which shortly before had been obtained by Willstätter,<sup>3</sup> by the action of ammonia upon  $\alpha$ - $\delta$ -dibromopropyl-malonic ester. Since then it has been obtained by Fischer and Zemplén,<sup>4</sup> and also by Sørensen and Anderson,<sup>5</sup> by modifications of the phthalimido malonic ester method.

Further investigation has shown that this acid occurs among the decomposition products of a variety of proteins, including the protamines. It was thought at one time that  $\alpha$ -pyrrolidine carboxylic acid was not a primary product of hydrolysis, for it might be derived from  $\alpha$ -amino  $\delta$ -hydroxyvaleric acid, which readily passes into pyrrolidine carboxylic acid on treatment with acids. This hydroxy acid has not, however, been detected among the products of protein decomposition.

Moreover, Fischer and Bochner<sup>6</sup> were able to show that proline cannot be formed from the hydroxy acid by alkalis; but nevertheless obtained 7.6 per cent. of proline by hydrolysing gelatine with baryta.

$\alpha$ -Pyrrolidine carboxylic acid is readily soluble in alcohol, and can be partially separated by this solvent from most other amino acids. It is known in both the active and racemic forms.

Shortly after the discovery of pyrrolidine carboxylic acid, Fischer found a hydroxy derivative of the same acid among the products of hydrolysis of gelatine.<sup>3</sup> The isolation of the latter is a tedious and

<sup>1</sup> Ber., 1908, 41, 893.

<sup>2</sup> Zeit. physiol. Chem., 1901, 33, 167; 1902, 35, 227.

<sup>3</sup> Ber., 1900, 33, 1160.

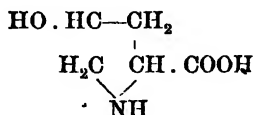
<sup>4</sup> Ber., 1909, 42, 1022.

<sup>5</sup> Zeit. physiol. Chem., 1908, 58, 236.

<sup>6</sup> Zeit. physiol. Chem., 1910, 65, 118.

<sup>7</sup> Ber., 1900, 33, 1160.

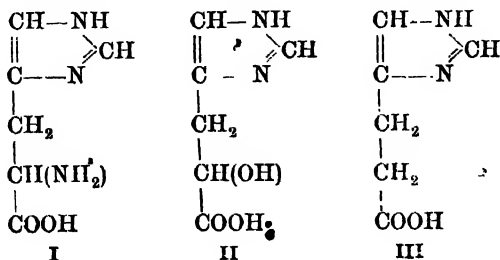
difficult process, and has only been accomplished in the case of a few proteins. It is a strongly laevorotatory substance, and on reduction is converted into pyrrolidine carboxylic acid. Two stereoisomeric hydroxypyrrolidine carboxylic acids, with the following structural formula,



have recently been synthesized by Leuchs,<sup>1</sup> and one of these probably corresponds to the racemic form of the optically active acid obtained by Fischer.

Histidine is an interesting amino acid which has been found among the products of hydrolysis of many different proteins. It was discovered by Kossel<sup>2</sup> among the decomposition products of a protamine named *sturine* (p. 160), and was obtained independently by Hedin<sup>3</sup> from the products of hydrolysis of the more complex typical animal and vegetable proteins. The quantity is usually small, but in some proteins, such as globin, the yield may be as high as 10 per cent.

Until recently histidine was classified among the diamino acids, but it is now known to have little in common with these substances. Histidine is, in fact, an iminazole derivative which accounts for the formation of a red colouring matter when it is treated with alkaline solutions of diazonium salts. The generally accepted formula for histidine (I) which was adopted by Pauly<sup>4</sup>, has received support from the experiments of Knoop and Windaus<sup>5</sup>, who showed that  $\beta$ -iminazole propionic acid (III) was formed by the reduction of the substance (II), obtained by the action of nitrous acid upon histidine.



*Ber.*, 1905, 38, 1937.

*Zeit. physiol. Chem.*, 1896, 22, 176.

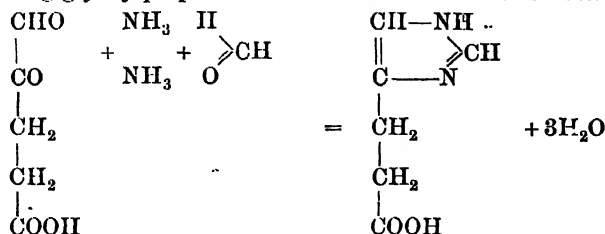
*Zeit. physiol. Chem.*, 1896, 22, 191.

*Zeit. physiol. Chem.*, 1904, 42, 508.

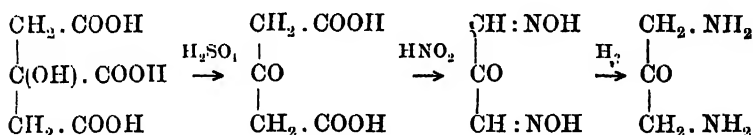
*Beitr. z. Chem. Phys. u. Path.*, 1905, 7, 144



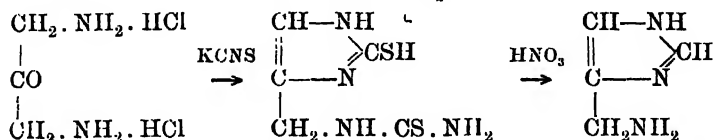
Knoop and Windaus synthesized  $\beta$ -iminazole-propionic acid by condensing glyoxylylpropionic acid with ammonia and formaldehyde.



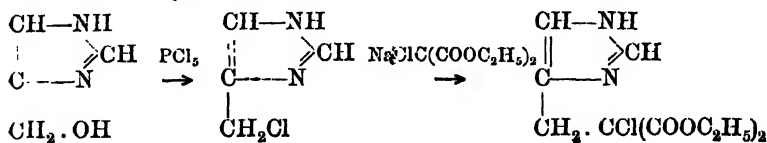
This view of the structure has been fully confirmed by the remarkable synthesis which was accomplished by Pyman<sup>1</sup> in 1911. The starting-point was citric acid, which was converted by well-known methods successively into acetone dicarboxylic acid, diisnitroso acetone and diamino acetone.



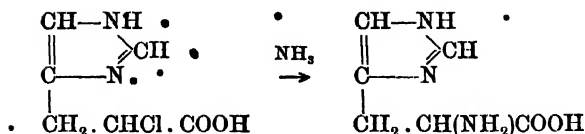
In the subsequent operation Gabriel's method of synthesising an iminazole ring was employed, and consists in acting on an amino-ketone with potassium thiocyanate and oxidising the product with nitric acid, whereby the thiol (SH) group is removed.



The product is then treated with nitrous acid, which replaces the amino group by hydroxyl, and the hydroxyl, when substituted by chlorine, enables the substance to combine with sodium chloromalonate ester. The product is hydrolysed, carbon dioxide removed, and the chlorine atom replaced by  $\text{NH}_2$ .



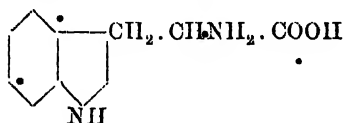
<sup>1</sup> *Trans. Chem. Soc.*, 1911, 99, 672, 1892, 2172; 1916, 109, 186.



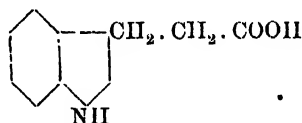
The product was finally resolved into its active constituents by fractional crystallisation with tartaric acid. The laevo compound is identical with the natural substance.

Tryptophane, which was first isolated from casein by Hopkins and Cole,<sup>1</sup> contains an indole nucleus, and therefore presents certain analogies with the aromatic as well as with the heterocyclic acids. Though one of the most constant constituents of the proteins, it is not found in most of the protamines nor in gelatine.

Some knowledge of the structure of tryptophane is derived from the fact that, on fusion with potash, it gives both skatole and indole, and a similar decomposition is brought about by putrefactive organisms which yield, in addition, indole-acetic acid and indole-propionic acid (II). Tryptophane has been synthesised, and its reactions agree well with the following formula (I):



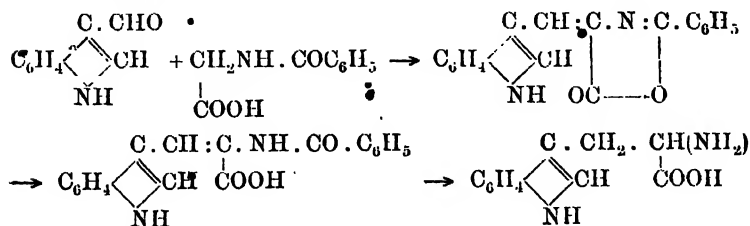
I. Tryptophane.



II. Indole-propionic acid.

That the amino group is probably in the  $\alpha$ -position, follows from the observation of Hopkins, that optical activity disappears when tryptophane is converted into indole-acetic acid.

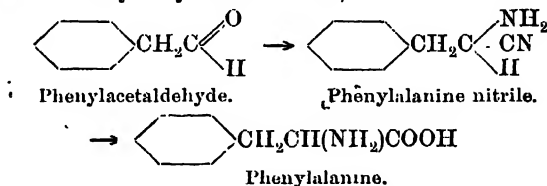
This view has been fully confirmed by its synthesis by Ellinger and Flaman<sup>2</sup> from  $\beta$ -indole aldehyde, which is condensed with hippuric acid in presence of sodium acetate, and acetic anhydride; the lactone is obtained, which is hydrolysed with boiling sodium hydroxide solution and then reduced with sodium in alcohol, which at the same time hydrolyses the benzoyl group.



Tryptophane, either in the free state or when combined in the protein molecule, is readily detected by the deep violet-blue colour which it yields on treatment with strong sulphuric acid and a trace of glyoxylic acid. A violet colour results on the addition of a small quantity of chlorine or bromine to an acid solution of tryptophane.

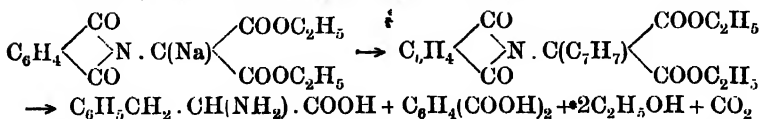
*The Aromatic Amino Acids (Phenylalanine and Tyrosine).* Phenylalanine ( $\alpha$ -amino- $\beta$ -phenylpropionic acid) was found by Schulze<sup>1</sup> more than twenty-five years ago in plant seedlings, and in the products of hydrolysis of seed proteins; but it is only within recent years, with the aid of the ester methods of isolation, that phenylalanine has been recognized as a common constituent of the typical proteins. Phenylalanine in most respects resembles the aliphatic monamino acids. On oxidation with potassium dichromate and sulphuric acid it yields phenylacetaldehyde, which is readily detected by its hyacinth-like odour. It is interesting to note that phenylalanine, under the influence of bacteria yields phenylethylamine, phenylacetic acid, and phenylpropionic acid, changes which closely resemble those sustained by tyrosine and tryptophane under similar conditions.

Phenylalanine was first synthesised by Erlenmeyer and Lipp<sup>2</sup> as follows: phenylacetaldehyde is converted into the nitrile of phenylalanine by the action of ammonia and hydrocyanic acid, and yields phenylalanine on hydrolysis with acids,



A more convenient synthesis from benzylmalonic acid has been described by Fischer,<sup>3</sup> who has also resolved the racemic acid into its optically active components.

A third method described by Sørensen<sup>4</sup> consists in combining potassium phthalimide with bromomalonic ester. This gives a sodium derivative in which the metal is replaced by benzyl, and the free acid is then hydrolysed, phthalic acid being separated and carbon dioxide removed from the malonic acid derivative.



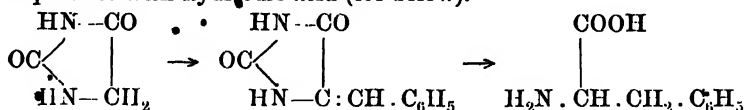
<sup>1</sup> E. Schulze and Bosshard, *Ber.*, 1881, 14, 1785; *Zeit. physiol. Chem.*, 1884, 9, 63

<sup>2</sup> *Ber.*, 1882, 15, 1006.

<sup>3</sup> *Ber.*, 1900, 33, 2383; 1904, 37, 3064.

<sup>4</sup> *Zeit. physiol. Chem.*, 1905, 42, 448.

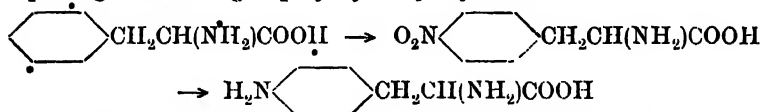
Wheeler and Hoffmann<sup>1</sup> have prepared it by condensing benzaldehyde with hydantoin and reducing and at the same time hydrolysing the product with hydriodic acid (see below).



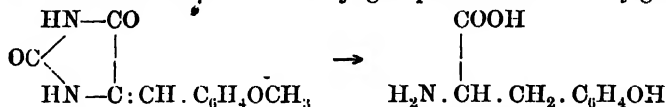
Tyrosine ( $\alpha$ -amino- $\beta$ -hydroxyphenyl-propionic acid) was one of the earliest known protein derivatives, and, on account of the ease with which it may be detected, its presence has been established in almost all proteins with the exception of gelatine and some of the protamines.

Tyrosine is readily detected by Millon's reagent,<sup>2</sup> which gives a red coloration or precipitate on warming. This reaction is due to the phenolic group and is shared by other phenols, but among the protein derivatives no substance other than tyrosine reacts in this manner. The test is directly applicable to proteins as well as to free tyrosine.

The synthesis of tyrosine has been accomplished by nitrating phenylalanine, reducing the resulting *para*-nitro derivative, and then replacing the amino group by hydroxyl by means of nitrous acid.<sup>3</sup>



It has also been obtained by Erlenmeyer's method from *p*-hydroxybenzaldehyde and hippuric acid, and by Wheeler and Hoffmann from anisaldehyde and hydantoin. The action of hydriodic acid in the last stage reduces and hydrolyses the condensation product and at the same time removes the methyl group from the methoxy group.

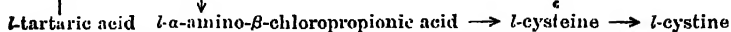
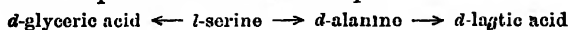


In concluding this short survey of the synthesis of the protein cleavage products, the interesting point arises as to the relation of the optically active forms, and these again to the active sugars and other natural products, seeing that they all occur together in the organism and are probably elaborated or decomposed by a similar mechanism. An attempt has been made to answer this question by Fischer and his co-workers by converting the active products into one

<sup>1</sup> Amer. Chem. J., 1911, 45, 368.

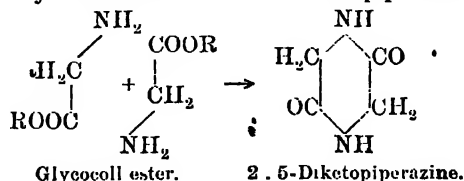
<sup>2</sup> A solution of mercuric nitrate containing nitrous acid. <sup>3</sup> Ber., 1882, 15, 1544.

another.' Supposing no optical inversion to occur, the following scheme represents the relationships:



**Esters of the Amino Acids.** The amino acid esters have acquired great importance, not only on account of their employment in the synthesis of *polypeptides* and other amino acid derivatives, but also owing to their great practical value as a means of separating the acids from mixtures produced by protein hydrolysis. The esters were originally prepared by the action of alkyl iodides upon the acids, but this method has a limited application and is no longer used. Curtius showed that the amino acids were readily converted into the hydrochlorides of their esters by the action of hydrochloric acid and alcohol, and in some cases the free esters were obtained from the salts by treatment with silver oxide. A more practical method for the isolation of most of the esters is that devised by Fischer,<sup>2</sup> who found that the crude hydrochlorides can be decomposed at a low temperature with caustic soda and may then be extracted by ether from the solution, after saturation with potassium carbonate. Another method employed by Fischer of separating the esters from their hydrochlorides is to add the calculated quantity of sodium ethoxide in alcoholic solution, whilst Levene<sup>3</sup> uses barium hydroxide in place of caustic soda. The free esters may be purified by distillation under low pressure.<sup>4</sup> Some amino acids, e. g. tyrosine, require special methods for the liberation of their esters, while in the case of the esters of histidine and the diamino acids it is impossible to effect purification by distillation.

The esters are strongly basic liquids, with a peculiarly unpleasant smell. They form crystalline salts with acids and are readily hydrolysed by water or alkalis. They are very reactive, unstable substances, and are converted into diketopiperazine derivatives on long standing, or better, by heating to 100°. Glycocoll ester, for example, is readily transformed into 2.5-diketopiperazine.



<sup>1</sup> Ber., 1907, 40, 1057, 3717; 1908, 41, 893; Freudenberg, Ber., 1914, 47, 2027.

<sup>2</sup> Ber., 1901, 34, 433.

<sup>3</sup> Levene and van Slyke, J. Biol. Chem., 1909, 6, 419.

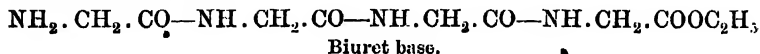
<sup>4</sup> E. Fischer, Ber., 1901, 34, 433.

It is possible to effect a partial separation of the esters by fractional distillation. The investigation of the products of hydrolysis of the proteins, so far as it concerns the *mono*-amino acids, is accomplished by boiling the protein with strong hydrochloric acid, evaporating under diminished pressure, and then treating the residue with alcohol and hydrogen chloride. The free esters are extracted as described above and are then distilled *in vacuo*. The different fractions are then hydrolysed and examined for the corresponding amino acids.<sup>1</sup> The boiling-points of some of the more important amino acid esters are given in the following table:

<i>Ethyl Ester.</i>	<i>b. p.</i>	<i>Pressure in mm.</i>
Glycine	51.5°-52.5°	10
Alanine	48.5	10
<i>dl</i> -Valine	63.5	8
Leucine and Proline	83.5	12
<i>l</i> -Aspartic acid	126.5	11
<i>d</i> -Glutamic acid	139-140	10
<i>dl</i> -Phenylalanine	143	10

**Polypeptides.** The fact that the proteins break up on hydrolysis by acids and alkalis into amino acids renders it probable that they are united in the form of amides in which the carboxyl group of one amino acid is linked to the amino group of another, and this view is supported by the fact that nitrous acid evolves little free nitrogen (no free amino group is therefore present), and that the proteins give the biuret reaction, a reaction peculiar to those substances which contain amide groupings.

The first systematic attempt to link together in chains a series of amino acids in this way was made by Curtius.<sup>2</sup> He found that glycine ester, carefully separated from its hydrochloride and dissolved in dry ether, deposited a solid substance on standing which gave the biuret reaction and hence was termed biuret base. It proved to be triglycylglycine ester.



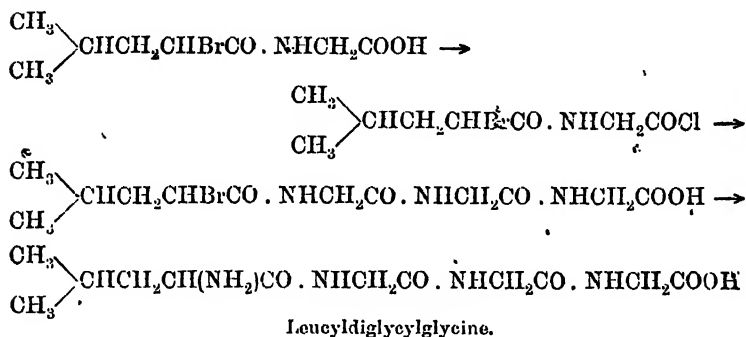
Later, he prepared mixtures of benzoyl glycylglycines containing as many as six glycyl radicals by heating together benzoyl chloride and

<sup>1</sup> The practical details of these operations will be found in papers by Fischer and others in the *Zeit. physiol. Chem.*, vol. 83 et seq., and in *The Chemical Constitution of the Proteins*, Part I, by R. H. A. Plimmer, Longmans, 1912.

<sup>2</sup> *Ber.*, 1904, 37, 1284.

from which the amide can be obtained by hydrolysis, but there is no means of removing the carbethoxyl group. If, however, the end amino group is protected by uniting it to a halogen acid chloride the amino acid may then be converted into the acid chloride by the action of phosphorus pentachloride, using acetyl chloride as solvent. The halogen in the halogen acid chloride is treated with ammonia after union with the new amino acid has been effected.

For example,  $\alpha$ -bromisocaproylglycine, treated with phosphorus pentachloride and acetylchloride, is converted into an acid chloride from which a number of polypeptides may be prepared. Thus, on condensation with glycylglycine ester it forms a product which gives the tetrapeptide, leucyldiglycylglycine, on hydrolysis and subsequent treatment with ammonia.



It will be readily understood, from the examples given above, that an almost infinite number of polypeptides can be obtained by combining various amino and diamino acids in their active and inactive forms. Among the compounds prepared in this way Fischer has obtained a polypeptide with as many as 18 amino acids linked together in a chain. As a class, they show a close resemblance to the natural peptones; the majority are soluble in water; with the exception of some of the di- and tri-peptides, they give the biuret reaction; they are precipitated by phosphotungstic acid; they have a bitter peptone-like taste and are readily hydrolysed by acids, and in many cases by trypsin, forming amino acids (see p. 77). The closest resemblance to the natural peptones and proteoses is found in those polypeptides which have a long chain composed of different amino acid radicals.

**Protein Derivatives, Proteoses and Peptones.** The proteoses and peptones are substances formed by the partial hydrolysis of

proteins, and their relation to the proteins may be compared to that existing between the di- and tri-saccharoses or the dextrans and starch. They are commonly prepared by the peptic digestion of proteins. After removal of any unchanged coagulable protein, the different proteoses are fractionally salted out with ammonium sulphate.<sup>1</sup> After complete saturation with ammonium sulphate the filtrate which contains the peptones is evaporated, freed as far as possible from ammonium sulphate, and the peptones precipitated with alcohol. As will be seen from the mode of preparation, the proteoses and peptones are distinguished by their different solubilities in salt solutions. They may be further identified by the biuret test and the action of nitric acid. The proteoses give a reddish-violet biuret reaction, and are precipitated by dilute nitric acid in the cold, especially in presence of sodium chloride; the peptones give an intense pink biuret reaction, and are not precipitated by nitric acid nor by many protein precipitants.

The peptones have a lower molecular weight than the proteoses, but exact determinations are in most cases still wanting. They are readily diffusible substances, and in this respect show a great contrast to the proteoses, which diffuse but slightly, and to the proteins, which are non-diffusible.

A great many different peptones have been described, but the methods employed for their isolation are such as to lead to the belief that most of them represent mixtures of closely related bodies rather than individual substances.

In recent years, by a process of graduated hydrolysis through the combined action of enzymes and chemical reagents, Fischer and others have succeeded in isolating a number of the simpler proteoses or peptones belonging to the class of polypeptides described above. Thus, in conjunction with Abderhalden,<sup>2</sup> he obtained from silk-fibroin a tetrapeptide consisting of two molecules of glycine, one of *l*-tyrosine, and one of *d*-alanine; Levene and Beatty<sup>3</sup> discovered glycyL-proline anhydride among the products of the tryptic digestion of gelatine; Osborne and Clapp<sup>4</sup> a compound of phenylalanine and proline by the acid hydrolysis of gliadin (p. 164); and Abderhalden,<sup>5</sup> by partial

<sup>1</sup> The proteoses according to Kühne's classification are divided into primary (protalbumose and hetero-albumose) and secondary proteoses, and it was believed that the primary proteoses on further hydrolysis were converted into secondary proteoses and eventually into peptones. The task of separating the different proteoses has been attempted by many chemists, but cannot be referred to here. It is improbable that the substances obtained were chemical individuals.

<sup>2</sup> *Sitzungsber. R. Akad. Wiss. Berlin*, 1907, 30, 574.

<sup>3</sup> *Ber.*, 1906, 30, 2060.

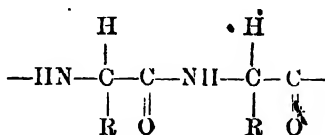
<sup>4</sup> *Amer. J. Physiol.*, 1907, 13, 123.

<sup>5</sup> *Zeit. physiol. Chem.*, 1909, 58, 373, 02, 315.



hydrolysis of edestin from cotton-seed, several di- and tri-peptides containing glutamic acid and tryptophane together with leucine, one containing tyrosine, glycine, and leucine, and many others. Attempts have been made to prepare these compounds synthetically. Fischer,<sup>1</sup> for example, obtained glycyl *d*-alanyl glycyl *l*-tyrosine, and glycyl *l*-tyrosine glycyl *d*-alanine, which contain the four amino acids of the tetrapeptide of silk-fibroin, but they are not identical with the natural product, a result which may be due to a wrong arrangement of the amino acid radicals or to the product being a mixture. The dipeptide of Osborna and Clapp was, however, obtained by uniting *l*-prolyl chloride with *l*-phenylalanine ester and hydrolysis of the resulting ester. *Metaproteins* include substances such as acid and alkali albumins which are formed by the action of acids and alkalis on certain proteins. Little is known of the structure or relation of these two substances.

**The Classification of the Proteins.** Many different schemes of classification have been proposed, but, owing to our ignorance of the constitution of the proteins, they are all more or less artificial. It is generally believed that the amino acids, which constitute the products of protein hydrolysis, are linked together in the protein molecule, as in the polypeptides, by the union of the amino group of one molecule with the carboxyl group of another. In the case of aliphatic monamino acids the type of grouping may be represented as follows:



Even if it is assumed that this simple type of amino acid condensation is the only one present in the protein molecule, it is clear that the combination of the different types of amino acids, obtained on hydrolysing proteins (p. 135), must give rise to extremely complex systems. There is, however, no reason to suppose that other types of grouping may not be present. E. Fischer suggests the possibility of the presence of piperazine groups in the protein molecule, ring formations of this character being easily accounted for by the elimination

<sup>1</sup> *Ber.*, 1903, 41, 850, 2860.

of water from a complex of two amino acid molecules, as follows:



It is also possible that the hydroxyl groups in the hydroxy-amino acids are not present in the original protein molecule, but are derived from anhydrides formed by intramolecular condensation.

As previously stated, the extremely limited extent of our knowledge of the molecular structure of the proteins renders a scientific system of classification at present impossible. Nevertheless a system may be devised which is based, not on the properties of the individual proteins, but on their products of hydrolysis. For example, the protamines, a group of basic proteins obtained from fish spermatozoa, commonly yield more than 80 per cent. of diamino acids, and only small quantities of monamino acids, whereas the majority of more complex proteins yield relatively little diamino acids and a large proportion of monamino acids. (An idea of the composition of the products of hydrolysis of some types of proteins may be obtained from the accompanying table (p. 158).) Unfortunately the data concerning the nature and proportion of the various amino acids, derived from different proteins, are insufficient to serve as the sole basis for a system of classification. But there is an additional fact upon which an arrangement may be based. Many complex proteins on gentle hydrolysis are resolved into two portions, one essentially protein and the other non-protein in character. An example of such a substance is furnished by the blood-pigment, *oxyhaemoglobin*, which is readily resolved into a protein substance, *globin*, and an iron-containing non-protein substance, named *haematin*. These complex proteins which contain a non-protein portion are termed *conjugated proteins*, whilst the groups in the original molecule which yield the non-protein substance are termed *prosthetic groups*. It is therefore possible to sub-divide some of the proteins into different classes according to the nature of their prosthetic groups.

The following scheme for the division of the proteins into six main classes must be considered as a convenient rather than a strictly scientific arrangement.

- (1) Protamines.
- (2) Histones.

Name of Protein.	Class of Protein.	Glycine.	Alanine.	Valine.	Leucine.	Aspartic acid.	Glutamic acid.	Serine.	Cysteine.	Lysine.	Arginine.	Proline.	Oxyproline.	Histidine.	Tryptophane.	Phenylalanine.	Tyrosine.
Salmine	Protamine	0	0	4.5	0	0	0	7.8	0	0	87.4	11.0		0	0	0	0
Thymus histone	Histone						3.65			7.7	14.36			1.21			6.31
Egg albumin	Albumin	0	2.22	2.5	10.71	2.20	9.10	?	?	3.76	4.91	3.36		1.71	+	5.07	1.77
Edestin (from hemp seeds)	Globulin	3.8	3.6	+	20.9	1.25	18.8	0.33	0.25	1.0	11.7	4.1	2.0	1.1	+	3.1	2.11
Glialin (from wheat)	Glialin	.02	2.0	0.2	5.61	0.58	37.33	0.13	0.45	0	3.16	7.06		0.61	+	2.35	1.2
Haemoglobin	Chromoprotein	0	4.19		30.0	4.43	1.73	0.56	0.81	4.28	5.42	2.34	1.04	10.96	+	4.54	1.53
Caseinogen	Phosphoprotein	0	1.5	7.2	9.4	1.1	15.6	0.5	+	6.0	3.8	6.7	0.3	2.5	1.5	8.2	4.5
Keratin (from horn)	Scleroprotein	0.31	1.20	5.70	13.80	2.50	3.00	0.68	7.0	+	+	2.00				3.00	4.58

- (3) Albumins and globulins.
- (4) Glutelins and gliadins.
- (5) Conjugated proteins (nucleoproteins, chromoproteins, glucoproteins).
- (6) Phosphoproteins.
- (7) Unclassified proteins (scleroproteins).
- (8) Protein derivatives (metaproteins, proteoses, peptones, polypeptides).

A special description of the general properties of each of these classes of substances, except group 8 which has already been discussed, is found in the following pages, together with a few details of some of the more important individual substances.

**The Protamines and Histones.** The protamines<sup>1</sup> comprise a small number of proteins which have a very limited distribution. They are far simpler in constitution than such proteins as egg-albumin, and their properties show corresponding differences. They occur in combination with nucleic acids (p. 165) in the form of nucleoproteins, which constitute practically the whole of the head or nucleus of the spermatozoa of certain kinds of fish.

These substances were first investigated in 1874 by Miescher, who obtained an impure protamine from salmon testicles, but, although he accurately determined the chief characteristics of the base, these early observations were overlooked. The detailed study of the protamine group is almost entirely due to Kossel and his pupils.

The protamines are found only in the spermatozoa of a few kinds of fish, among which the salmon, herring, sturgeon, mackerel, and carp are the most important. They are prepared by extracting the ripe spermatozoa with dilute sulphuric acid and precipitating the filtered solution with alcohol. In this way a crude protamine sulphate is obtained as a white flocculent precipitate which is only moderately soluble in water. On concentrating a solution of the protamine sulphate, a clear, colourless oil settles out, which contains most of the sulphate. This oil can be separated and the protamine further purified by conversion into the picrate.

The protamines are strongly basic substances which absorb carbon dioxide from the air, and form fairly well-defined, sparingly soluble salts with platinic chloride, cupric hydrate, and silver oxide. Their aqueous solutions are not coagulated by boiling, but precipitates are

<sup>1</sup> A complete account of the protamines together with the whole of the literature will be found in a 'Sammel-Referat', Kossel, in the *Biochemisches Centralblatt*, 1906, v, pp. 1 and 33.

formed by the usual protein precipitants, such as phosphotungstic, chromic and picric acids, and potassium ferrocyanide.

The proportion of nitrogen (25–30 per cent.) in the protamines is higher than in any other class of proteins, but they contain neither phosphorus nor sulphur. With the exception of cycloptertine and cyprinine the protamines do not react with Millon's reagent, and tyrosine is not found among their products of hydrolysis. Cycloptertine gives the Adamkiewicz reaction, but the tryptophane complex is uniformly absent from the other members of the group.

With one exception they all yield remarkably large amounts of arginine on hydrolysis, and this fact no doubt accounts for their strongly basic properties. The following table<sup>1</sup> contains the main results of these investigations.

*Hydrolytic Products of the Protamines.*

Source of Protamine.	Name of Protamine.	Arginine.	Lysine.	Histidine.	Alanine.	Serine.	Amino-valeric acid.	Proline.	Leucine.
Mackerel	Scombrine	88.8	—	—	6.8	—	—	3.8	—
Salmon	Salmine	87.4	—	—	—	3.2	1.6	4.3	—
Herring	Clupeine	89.0	—	—	+	+	+	+	—
Sturgeon	Sturine	63.1	8.4	11.8	+	—	—	—	+
Carp	Cyprinine	8.6	30.3	—	?	?	+	?	?

It will be seen that among the products of the hydrolysis of salmine, clupeine, and scombrine, about 88–89 per cent. of the total nitrogen is found as arginine, and a further 10 per cent. is present in the form of monoamino acids (alanine, serine, aminovaleric acid and proline), but lysine and histidine are absent.<sup>2</sup> Thus the products of hydrolysis, which have been identified, represent almost the whole of the original protamine. This is a matter of some interest, because in the case of the more complicated proteins it has so far been impossible to account for more than about 70 per cent., and frequently very much less of the original material.

Salmine, which has been more completely investigated than the other protamines, consists approximately of 10 mols. arginine, 2 mols. serine, 1 mol.  $\alpha$ -aminovaleric acid, and 2 mols. proline.

<sup>1</sup> The numbers express the percentage of nitrogen present as amino acids compared with the total nitrogen.

<sup>2</sup> The details of these methods, which depend essentially upon the varying properties of the silver salts of histidine, arginine, and lysine, will be found in papers by Kossel and his pupils in the *Zeit. physiol. Chem.* (Cf. especially Kossel and Kutscher, 1900. 31, 165.)

These results are in agreement with a formula such as  $C_{81}H_{155}N_{45}O_{18}$  with a minimal molecular weight of 2045. Although in the present state of our knowledge this formula can obviously have no claim to exactness, it at least indicates the extreme complexity of even the simpler proteins.

By the regulated action of acids and enzymes, it has been possible to prepare substances intermediate between the protamines and amino acids. These substances have been called *protones*, and the name suggests a relationship to the protamines which may be regarded as similar to that existing between proteins and peptones. The protones give the biuret reaction and resemble the simple polypeptides in most respects; up to the present, however, little progress has been made in the effort to obtain them in a well-defined form.

Faintly alkaline solutions of the protamines yield precipitates with coagulable proteins and certain proteoses which bear the closest resemblance to the naturally occurring *histones*. The latter are substances of great biological significance, and, like the protamines, are found in combination with nucleic acids. The more important histones have been prepared from the nucleated red blood-cells of birds,<sup>1</sup> from certain animal glands, especially the thymus,<sup>2</sup> and from the spermatozoa of the cod, mackerel, sea-urchin,<sup>3</sup> and certain other fish.<sup>4</sup> It appears that the unripe testicles of all fish contain histones, but in a few cases the process of ripening is accompanied by a conversion into protamine, although generally the histone persists.

The histones are distinctly basic substances with a high percentage of nitrogen (17-20 per cent.). Their properties vary considerably according to the source from which they are derived, and, as a consequence, the group as a whole is not very clearly defined. The histones show resemblances to the protamines on the one hand, and to the coagulable proteins and proteoses on the other. In most cases their solutions are not coagulated on heating, except in the presence of salts, and their basic character is inferred from the fact that most of them are precipitated by ammonia. On hydrolysis, the histones yield a larger proportion of basic products than the typical coagulable proteins, and this is clearly in harmony with the view that they are to be regarded as occupying a position

Kossel, *Zeit. physiol. Chem.*, 1884, 3, 511.

Lillienfeld, *Zeit. physiol. Chem.*, 1894, 18, 473; Lawrow, *Zeit. physiol. Chem.*, 1899, 28, 388.

Matthews, *Zeit. physiol. Chem.*, 1897, 23, 399.

There are various special ways of obtaining the histones, but one common method is to extract the compound of histone and nucleic acid with water. The histone is liberated from the nucleic acid by means of hydrochloric acid and then precipitated by ammonia.

intermediate between the protamines and the proteins of the egg-albumin type. Thus, the histone prepared from the spermatozoa of the codfish (*Gadus*-histone) yields, on hydrolysis, 26.8 per cent. of its nitrogen in the form of arginine, although many typical proteins yield less than 10 per cent.

Globin, the main protein constituent of haemoglobin, exhibits many of the properties of the histones and may be considered as belonging to the group, although it only contains 20 per cent. of diamino acids, which consist mainly of histidine. It will be referred to later under haemoglobin (p. 167).

**The Albumins and Globulins.** Albumins and globulins are coagulable proteins forming the more important constituents of the majority of animal and vegetable tissues. They contain sulphur, little or no phosphorus, and, with the exception of a carbohydrate group, no other prosthetic group. They may be regarded as the most typical proteins. Although a large number of albumins and globulins are known, it will be impossible to do more than indicate their general properties and consider one or two selected substances in slight detail.

Albumins are specially characterized by their solubility in distilled water, and by the fact that they are salted out from solution much less readily than the globulins and most other proteins. Salts, such as magnesium sulphate and sodium chloride, fail to precipitate the albumins, but they are precipitated by complete saturation of their solutions with ammonium sulphate. The most important members of the group are egg-albumin and serum-albumin, both of which have been obtained in the form of well-defined crystals by salting out an acid solution with ammonium sulphate under certain special conditions.<sup>1</sup> It is, however, doubtful if the crystalline compounds so obtained are identical with the products as they occur in nature. More probably a very slight amount of alteration has taken place during crystallization, and the crystals represent a salt formed by the combination of the albumin and the acid used in the process. The chemical difference is apparently so slight that at present it has little practical importance. The products of hydrolysis of serum- and egg-albumin include the majority of amino acids, except glycine, and consequently all the typical protein tests are given by these albumins. Both, on hydrolysis, yield glucosamine in addition to the other products, and from egg-albumin it is said that as much as 10-11 per cent. is obtainable.<sup>2</sup> The vegetable albumins, e. g. leucosin and legumelin of seeds, though soluble and coagulable, are more easily precipitated by salts, as a rule, than the animal albumins.

<sup>1</sup> Hofmeister, *Zeit. physiol. Chem.*, 1889, 14, 163; F. G. Hopkins, *Journ. of Physiol.*, 1895, 23, 130.

The globulins, which are much more numerous than the albumins, are a very important class of substances. They are found in the blood, in most animal tissues, in eggs, and traces also in milk, and include a large number of vegetable proteins. As far as ultimate composition and products of hydrolysis are concerned, they can scarcely be distinguished from the albumins, but the globulins contain glycine and, in addition, the two classes exhibit very different solubilities. The globulins possess the remarkable property of dissolving in water containing small quantities of inorganic salts, but they are precipitated when the salt concentration falls below a certain level either by dilution or dialysis;<sup>1</sup> they are insoluble in pure water and dilute acids, but readily dissolve in alkaline solution, from which they may be precipitated by weak acids, so that they appear to be feebly acid substances; they are much more readily salted out from solution than the albumins, and, unlike the latter, they are precipitated by saturation with magnesium sulphate or by half-saturation of the solution with ammonium sulphate.

Serum-globulin, which is perhaps the most carefully investigated of the globulins, is prepared by adding an equal volume of saturated ammonium sulphate solution to blood-serum. It is an unstable substance, readily passing over into insoluble modifications which do not dissolve in dilute salt solutions. Serum-globulin is probably a mixture of many different globulins.

\* The globulins give the typical protein reactions, and their products of hydrolysis include all the common amino acids. Most of the globulins yield, on hydrolysis, a substance of a carbohydrate character, which, in the case of egg-globulin, has been identified as glucosamine.

Reference must be made to thyreoglobulin, the remarkable globulin of the thyroid gland. This substance, which was discovered by Baumann, has all the properties of serum-globulin, but contains a considerable though varying quantity of iodine.<sup>2</sup> The iodine appears to be contained in a prosthetic group, for on hydrolysis with acids or enzymes an iodine-rich substance (14.2 per cent.) named *iodothylin* is liberated. Both the globulin and *iodothylin* have remarkable pharmacological properties, possessing great curative value in cases of myxoedema and cretinism.

Some of the muscle proteins and a protein circulating in the

<sup>1</sup> A discussion of this and other properties of the globulins from the standpoint of physical chemistry will be found in the Royal Society Croonian Lecture for 1905 by W. B. Hardy, and *Journ. of Physiol.*, 1905, 33, p. 251; also J. Mellanby, *Journ. of Physiol.*, 1905, 33, p. 338.

<sup>2</sup> Oswald, *Zeit. physiol. Chem.*, 1899, 27, 14; 1901, 32, 121.



blood, named *fibrinogen*, show close analogies to the globulins. They have the common property of clotting at low temperatures and forming two insoluble proteins, *myosin* and *fibrin*. The muscle proteins have been investigated by Halliburton<sup>1</sup> and von Fürth,<sup>1</sup> whilst fibrin, on account of its connection with the phenomenon of blood-clotting, has attracted the attention of a large number of chemists.

Many vegetable globulins have been carefully studied, and our knowledge of these substances is in some cases more complete than of the animal proteins. The early investigations were made chiefly by Ritthausen,<sup>2</sup> and have been continued by Osborne<sup>3</sup> and others. Some of them occur, or have been obtained, in the crystalline form, and offer promising material for further investigation. Perhaps the most important members of this group are *cdestin*, which has been prepared from the seeds of hemp, flax, linseed, cotton, and from other sources, *excelsin* from the Brazil nut, and *phascolin* from the kidney-bean. The substances obtained from these various materials are all very similar and closely resemble the animal globulins.

**The Glutelins and Gliadins.** This group of proteins have a vegetable origin and are usually present in the seeds of different plants. The glutelins are insoluble in neutral aqueous solutions, in saline solutions, and in alcohol. They yield on hydrolysis 12-20 per cent. of glutamic acid and 5-10 per cent. of arginine and leucine. The gliadins (gliadin, hordein, and zein) are soluble in alcohol, but not in water, though their salts with acids and alkalis dissolve readily. They are found in the seeds of all cereals. *Gliadin* of wheat and rye and *hordein* of barley contain more glutamic acid and less arginine than the glutelins. *Zein* is obtained from maize and contains more leucine and less glutamic acid than the other gliadins.

**The Conjugated Proteins.** The proteins are more complex substances than any of the proteins which have so far been considered. They are resolved by the action of acids or enzymes into two parts; one of these constituents is either a protamine, a histone, or a still

<sup>1</sup> Halliburton, *Journ. of Physiol.*, 8, p. 133; von Fürth, *Arch. exper. Path. u. Pharm.*, 1895, 36, 231.

<sup>2</sup> H. Ritthausen, *Die Eisskörper der Getreidreacen, Hülsenfrüchte und Oelsumen*, Bonn, 1872.

<sup>3</sup> T. E. Osborne. Many papers in *Amer. Chem. Journ.*, *Journ. Amer. Chem. Soc.*, and *Amer. Journ. of Physiol.*, from 1892, and *The Vegetable Proteins*, Monographs on Biochemistry. Longmans, 1909.

more complex protein; the other may have a widely varying nature and is known as the *prosthetic group*.

The proteins may be divided, according to the character of these groups, into three main classes—nucleoproteins, chromoproteins, and glucoproteins.

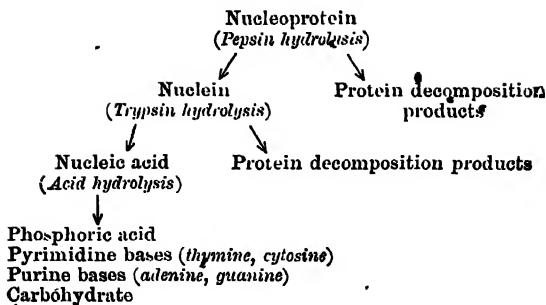
Nucleic acids represent the prosthetic group in the first class of substances; they are characterized by the presence of a large amount of phosphorus, and yield, on hydrolysis, many interesting products. The prosthetic group in the chromoproteins is represented by coloured substances, while the glucoproteins contain carbohydrate groups.

*The Nucleoproteins.* The simplest nucleoproteins are found in the spermatozoa of fish and have been carefully investigated by Miescher, Schmiedeberg, and especially by Kossel.<sup>1</sup> They may be regarded as salts formed by the union of a basic protein, which is either a protamine (p. 159) or histone (p. 159), with nucleic acids. This compound of protein and nucleic acid may represent as much as 96 per cent. of the dry matter in the head, or nuclear portion, of the spermatozoa. Nucleoproteins of a somewhat different type have been prepared from a very large number of animal tissues, and, of these, the preparations derived from the thymus and pancreas have been most carefully studied. The proteins in these nucleoproteins are usually much less basic than those obtained from spermatozoa. The practical details of the preparation of nucleoproteins from animal tissues leave it doubtful whether they are single individuals, and, as a consequence, the principal interest is transferred to the nature of their prosthetic groups.

When a nucleoprotein is carefully hydrolysed by pepsin, it is found that a portion of the protein is rapidly removed, leaving an insoluble substance, known as *nuclein*, which, however, still contains a certain amount of protein. On further hydrolysis, either with dilute acids or with trypsin, the remaining protein is removed, and nucleic acid and protein decomposition products remain in solution.

The nucleic acids are strongly acid substances which contain a high percentage of phosphorus. On hydrolysis with acids at high temperatures they yield a variety of interesting products, which include phosphoric acid, pyrimidine, and purine bases and carbohydrates. The successive stages in the hydrolysis of a nucleoprotein may be represented as follows:

<sup>1</sup> Miescher, *Arch. exper. Path.*, 1896, 37, 100; Schmiedeberg, *Arch. exper. Path.*, 1900, 43, 57; Kossel, many papers in *Zeit. physiol. Chem.*, and *Ber.* from 1893. A monograph by Burian on the chemistry of Spermatozoa in Ascher and Spiros' *Ergebnisse der Physiologie*, vol. iii, p. 48, 1904, should be consulted.

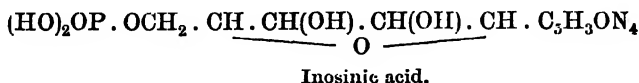
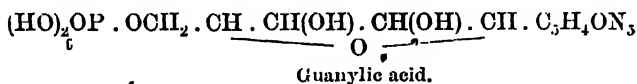


Among the pyrimidine bases, thymine and cytosine have been identified (p. 108).

The purine bases include adenine and guanine. The carbohydrate group has been definitely identified as *d*-ribose (p. 16).

A simpler form of nucleic acid, which is found in certain organs, yields on hydrolysis phosphoric acid, ribose, and guanine, and has been called *guanylic acid*, and another, known as *inosinic acid*, breaks up into the first two substances and hypoxanthine.

The structure of these and the more complex nucleic acids have been very fully discussed by Levene and Jacobs.<sup>1</sup> A simple nucleic acid composed of one molecule each of acid, base, and sugar they call a *mono-nucleotide*. Guanylic and inosinic acid belong to this class and are represented by the formulæ:

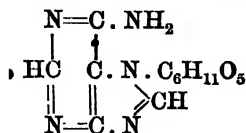


After splitting off phosphoric acid, the compound of sugar and base which remains is called a *nucleoside*. Guanosin is guanine-*d*-riboside and adenosin is adenine-*d*-riboside. A guanine hexoside has been obtained by Levene from thymus nucleic acid.<sup>2</sup> Such compounds have been recently prepared synthetically by Fischer,<sup>3</sup> by combining acetobromoglucose with the silver compounds of the bases (p. 47). Adenine glucoside, for example, has the probable formula:

<sup>1</sup> Ber., 1910, 43, 3150, 3164; 1911, 44, 746, 1027; Jones and Read, *J. Biol. Chem.*, 1917, 29, 111; Jones and Kennedy, *Abstr.*, 1919, i, 294.

<sup>2</sup> *J. Biol. Chem.*, 1912, 12, 378.

<sup>3</sup> Ber., 1914, 47, 210, 1058.



Adenine glucoside.

More recently Fischer<sup>1</sup> has succeeded in synthesising a definite nucleotide by introducing the phosphoric acid residue into theophylline glucoside by the action of phosphorus oxychloride in pyridine solution. It has the formulæ



The more complex nucleic acid derived from yeast is regarded as a combination of four nucleotides (probably linked as anhydrides of the phosphoric acid group) in which two pyrimidine and two xanthine bases are present.

*Chromoproteins.* Very few coloured protein substances are known, and of these, haemoglobin, the red colouring matter present in the blood of vertebrates, is the only one which is widely distributed. Haemocyanine is the main constituent of the blood of the octopus and similar animals, and resembles haemoglobin closely in many respects. It is an interesting fact that both these proteins contain metals in their prosthetic groups. Haemoglobin contains 0.47 per cent. of iron, whilst haemocyanine contains 0.38 per cent. of copper. A few other coloured proteins from lower types of organisms have been described, but they have not been fully investigated and will not be referred to here.<sup>2</sup> The chromogenic group in all coloured proteins is probably contained in the prosthetic group, and not in the protein part of the molecule.

*Haemoglobin.* Haemoglobin consists of a colourless protein, named *globin*, united to a coloured prosthetic group. Perhaps the most remarkable property that haemoglobin possesses is its ability to enter into combination with certain gases, among which oxygen, carbonic oxide, and nitric oxide are the most important. The product of the combination of haemoglobin and oxygen is known as *oxyhaemoglobin*. It was long ago obtained in the form of well-defined crystals, and has been the subject of numerous investigations. Many methods have been devised for its preparation, so that it can be obtained more readily and in a purer state than is possible with most of the other proteins.<sup>3</sup>

<sup>1</sup> Ber., 1914, 47, 3193.

<sup>2</sup> See *Vergleichende chemische Physiologie der niederen Tiere*, by von Fürth: Gustav Fischer, Jena, 1903.

<sup>3</sup> F. N. Schulz, *Zeit. physiol. Chem.*, 1898, 24, 449.

Both oxyhaemoglobin and haemoglobin crystallize in many different forms, almost all of which belong to the rhombic system, and are obtained from the blood of various animals, but, on repeated crystallization, the forms interchange, so that it is not certain that the different crystals represent differently constituted substances. Solutions of haemoglobin and its derivatives have been examined spectroscopically by a large number of investigators, and show well-defined absorption bands, details of which will be found in text-books on Physiological Chemistry.

The fact that haemoglobin solutions exert a distinct osmotic pressure has already been mentioned, and the question of its molecular weight, deduced both from osmotic pressure determinations and from the proportion of iron in the molecule, has also been discussed (p. 129).

A great many elementary analyses of haemoglobin have been made, and the following numbers are selected from closely agreeing analyses of horse haemoglobin by Hoppe-Seyler, and by Nencki:

$C = 54.8$ ;  $H = 7.0$ ;  $N = 17.2$ ;  $S = 0.65$ ;  $Fe = 0.47$ .

On gentle oxidation with potassium ferrieyanide, haemoglobin is converted into an interesting substance known as *methaemoglobin*. It has been obtained in crystals, and is isomeric with oxyhaemoglobin, but, unlike the latter substance, the oxygen cannot be removed by a stream of natural gas. It is found in the urine in disease, and may be identified spectroscopically. On reduction, it is reconverted into haemoglobin.

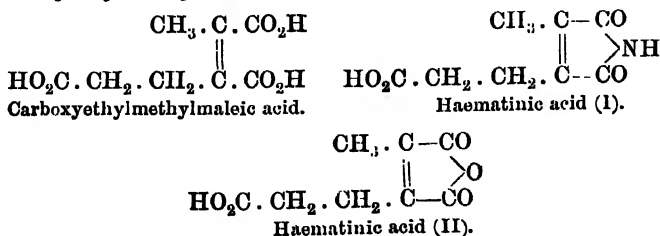
*The Resolution of Oxyhaemoglobin into Globin and Haematin.* The protein, globin, is very loosely combined with its prosthetic group, haematin, and the resolution of oxyhaemoglobin is brought about by very weak acids. This decomposition was observed as long ago as 1864 by Stokes and by Hoppe Seyler. More recent experiments have shown that oxyhaemoglobin may be decomposed by the action of acids, in the presence of alcohol and ether, in such a way that the colouring matter is dissolved by the ether, while the protein remains in the aqueous portion. The haematin is present in very much smaller quantity than the globin, as may be seen from the following approximate numbers<sup>1</sup>: globin = 94.1%; haematin = 4.5%. It is found that small quantities of ammonia and fatty acids of the acetic series are formed at the same time. The nature of the union between globin and haematin is quite unknown; but it is almost certain that it is not that of a simple salt.

<sup>1</sup> Lawrow, *Zeit. physiol. Chem.*, 1898, 26, 813.

Globin is a curious protein which has been shown by Schulz<sup>1</sup> to closely resemble the histones (p. 159). It is soluble in acids, but is precipitated by ammonia. It contains a high percentage of nitrogen (16.9 %), and, on hydrolysis, yields a large proportion of bases (histidine = 11 %, arginine = 5.4 %, lysine = 4.3 %). The amount of histidine is larger than that found in the products of hydrolysis of any of the common proteins. The other products of hydrolysis of globin<sup>2</sup> are given in the table on p. 158.

*Haematin and its Derivatives.* A large amount of work has been done by Küster, Piloty, H. Fischer, and Willstätter with the object of determining the nature of haematin; but its exact constitution is still obscure. Although the preparation of haematin in the pure state presents considerable difficulties, a crystalline substance, *haemin*, closely allied to it, is easily obtained. If a drop of blood is warmed on a microscope slide with glacial acetic acid and a trace of common salt, it is found, on cooling, that a quantity of small dark-brown plates and prisms separate out.<sup>3</sup> These crystals are composed of haemin, and are produced by the action of hydrochloric acid upon haematin, previously liberated from the haemoglobin by the acetic acid. A process for the preparation of large quantities has been devised by Schalféjew<sup>4</sup> which does not differ in principle from the micro-chemical method.

*Oxidation of Haematin.* The investigation of the oxidation products of haematin has been carried out by W. Küster and his pupils.<sup>5</sup> By acting upon haematin dissolved in acetic acid with sodium dichromate, two acids were obtained with the formulæ  $C_8H_9NO_4$  and  $C_8H_7O_5$ . Both of these substances were termed *haematinic acids*, and eventually proved to be the anhydride and imide respectively of carboxyethylmethylmaleic acid.



<sup>1</sup> *Zeit. physiol. Chem.*, 1898, 24, 449.

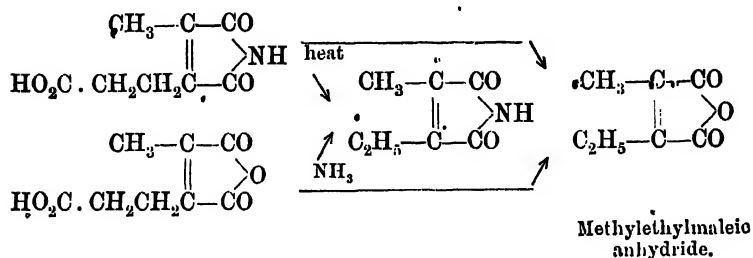
<sup>2</sup> Abderhalden, *Zeit. physiol. Chem.*, 1908, 37, 484.

<sup>3</sup> The formation of haemin crystals constitutes the best chemical test for the detection of blood and is used in conjunction with the spectroscopic method.

<sup>4</sup> *Chem. Centralbl.*, 1885, 18, 232.

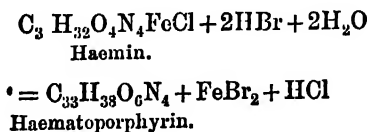
<sup>5</sup> *Annalen*, 1906, 345, 1, and earlier papers.

The structure of the haematinic acids follows from the fact that the imide,  $C_7H_9O_2N$ , obtained by heating haematinic acid (II) with ammonia at high temperatures, is identical with the synthetically prepared imide of methylethylmaleic acid, and the same product is also obtained by the dry distillation of haematinic acid (I). Both the haematinic acids have been converted into methylethylmaleic anhydride, and haematinic acid (II)<sup>1</sup> and methylmaleic anhydride have been synthesized. The changes may be represented as follows :



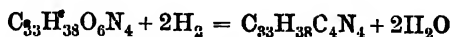
The relation between haemin and haematin is indicated to some extent by the following observations. Haematin is obtained from haemin by the action of caustic soda, and, on the other hand, haematin is converted into haemin by the action of hydrochloric acid; consequently, haemin is frequently spoken of as haematin hydrochloride. This, however, is incorrect, as apparently a hydroxyl group is replaced by chlorine. Moreover, if haemin is heated with aniline, hydrochloric acid is removed, a product named *dehydro-haematin* being formed which is not identical with haematin.

Haematin and haemin, as previously mentioned, contain a considerable quantity of iron, but this is readily removed by the action of strong acids, leaving an iron-free pigment named *haematoporphyrin*. The reaction may be represented as follows :



<sup>1</sup> Küster and Weller, *Ber.*, 1914, 47, 532; *Zeit. physiol. Chem.*, 1917, 99, 229.

Haematoporphyrin, on partial reduction with hydriodic acid, yields *mesoporphyrin*:



Haematoporphyrin.      Mesoporphyrin.

On further reduction with hydriodic acid, the mesoporphyrin is converted into a volatile compound, *haemopyrrole*,<sup>1</sup> which is probably a mixture of pyrrole homologues consisting mainly of two dimethyl ethyl pyrroles.

From recent researches of Willstätter and M. Fischer<sup>2</sup> it appears that a series of additive compounds of haemin with hydrogen bromide is first formed, which, on hydrolysis, yield haematoporphyrin. The latter substance has been obtained in crystals and its analysis and molecular weight correspond to the formula  $\text{C}_{33}\text{H}_{38}\text{O}_6\text{N}_4$ . With alcoholic potash in presence of pyridine it loses two hydroxyl groups and gives *haemoporphyrin*  $\text{C}_{33}\text{H}_{36}\text{O}_4\text{N}_4$ , from which, on distillation with soda-lime, two molecules of carbon dioxide are eliminated, giving *aetioporphyrin*,  $\text{C}_{31}\text{H}_{36}\text{N}_4$ . Aetioporphyrin is specially interesting, seeing that it is identical with the substance obtained from *aetiophyllin*, a degradation product of chlorophyll (see p. 173).

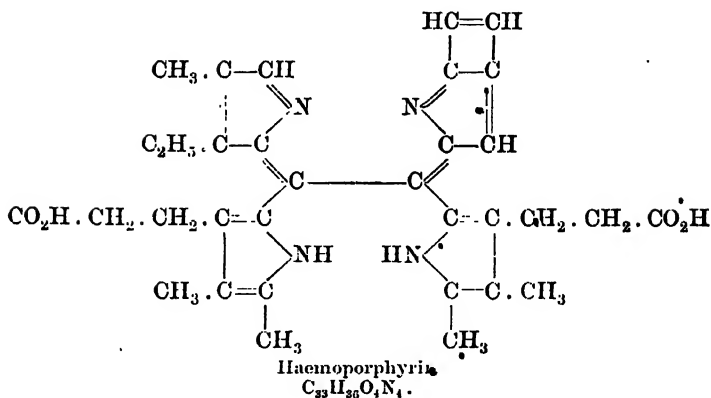
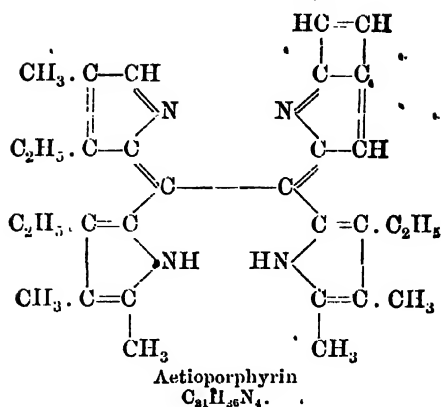
These substances are of the greatest interest, not only in connection with the structure of haematin and of chlorophyll, but also with that of bile and urinary pigments. Haematoporphyrin is found occasionally in the urine (especially after sulphonal poisoning, which produces considerable blood destruction) and is no doubt derived from haematin, set free from haemoglobin. Mesoporphyrin is probably identical with a substance described under the name of *haematoidin*, which was discovered by Virchow in 1847 in blood extravasations, and also with *bilirubin*, which is one of the best-known bile-pigments.

The relation of haemoporphyrin is that of a dicarboxylic acid of aetioporphyrin, whereas haematoporphyrin is regarded as a dihydroxy haemoporphyrin.

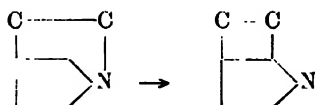
<sup>1</sup> These and other allied investigations by Nencki and his pupils are published in many different journals, but all will be found in Nencki's Collected Works (*Gesammelte Arbeiten von M. Nencki*, 2 vols. Vieweg & Sohn, Braunschweig, 1905).

<sup>2</sup> *Zeit. physiol. Chem.*, 1913, 87, 423.

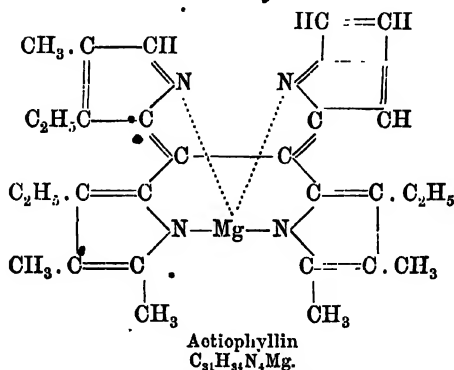
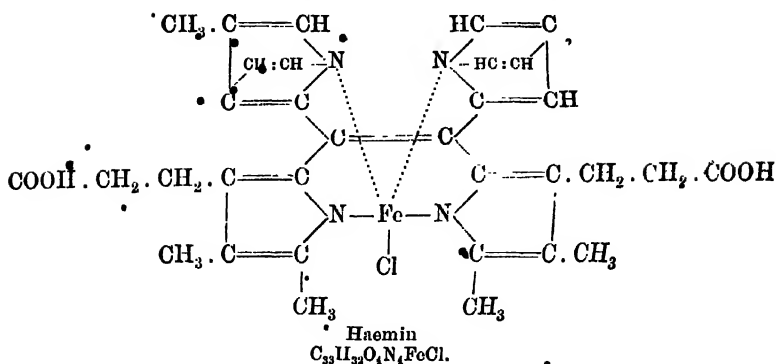




Now it appears that iron cannot be eliminated from haemin until hydrogen bromide has been introduced into the molecule, and this is explained by a change from a nitrogen to a carbon linking thus :



The formula proposed for haemin explains the production of an additive compound with two molecules of hydrogen bromide, the loosening of the bridged nitrogen ring, and the subsequent formation of a dihydroxy-dicarboxylic acid.



Alongside the formula for haemin is placed the one suggested for actiophyllin, a degradation product of chlorophyll<sup>1</sup> (see below).

**Chlorophyll.** As we have just seen, certain of the decomposition products of chlorophyll bear a close relationship to those of haemoglobin, and a brief account of this interesting colouring matter is therefore appended. Chlorophyll has been the subject of many investigations by Hoppe-Seyler, Schunck and Marchlewski, Nencki, and more recently by Willstätter and Stoll, whose brilliant researches have now been collected in book form and constitute one of the great classics in the domain of structural organic chemistry.<sup>2</sup>

Chlorophyll, which is apparently identical in all plants, consists of two crystalline substances, chlorophyll *a*, which gives a bluish-green,

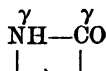
<sup>1</sup> Willstätter and Fischer, *Annalen*, 1911, 382, 129; 1913, 400, 147.

<sup>2</sup> *Untersuchungen über Chlorophyll*, by R. Willstätter and A. Stoll. Springer Berlin, 1913.

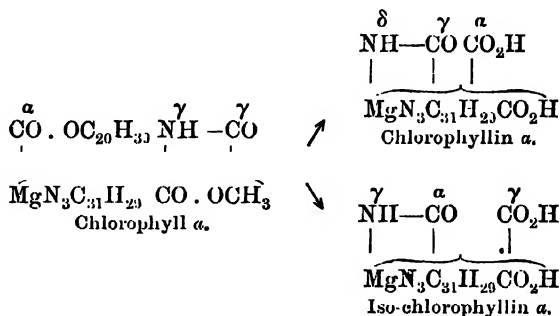
and chlorophyll *b*, a yellowish-green solution. They may be fractionally separated by using two solvents (methyl alcohol and petroleum ether), and each of these gives rise to a corresponding series of derivatives.

*Action of Alkalis on Chlorophyll.* The action of alkalis and acids is strongly differentiated. With alkalis, the chlorophylls, which are dicarboxylic esters of *phytol*,  $C_{20}H_{39}OH$ , and methyl alcohol, are hydrolysed. If the phytol is eliminated, the monomethyl ester which remains is termed *chlorophyllide*. On further hydrolysis, chlorophyllide yields green-coloured salts of acids, the *chlorophyllins* containing three carboxyl groups, one being present probably as a lactam group.

In the reaction with alkalis, chlorophyll passes through a brown phase, the green colour changing in the case of chlorophyll *a* to a yellowish-brown and in that of chlorophyll *b* to a red, when finally they return to the original green chlorophyll colour. This change is explained by the presence of a lactam group which opens and reforms in a new position. The original group may be marked :



It may undergo hydrolysis and the carboxyl  $\gamma$  enter into union with another nitrogen group  $\delta$ , or another carboxyl  $\alpha$  may combine with nitrogen  $\gamma$ . The hydrolysis takes place differently if carried out in the cold or with concentrated alkali at a higher temperature, and may be represented by the following scheme :

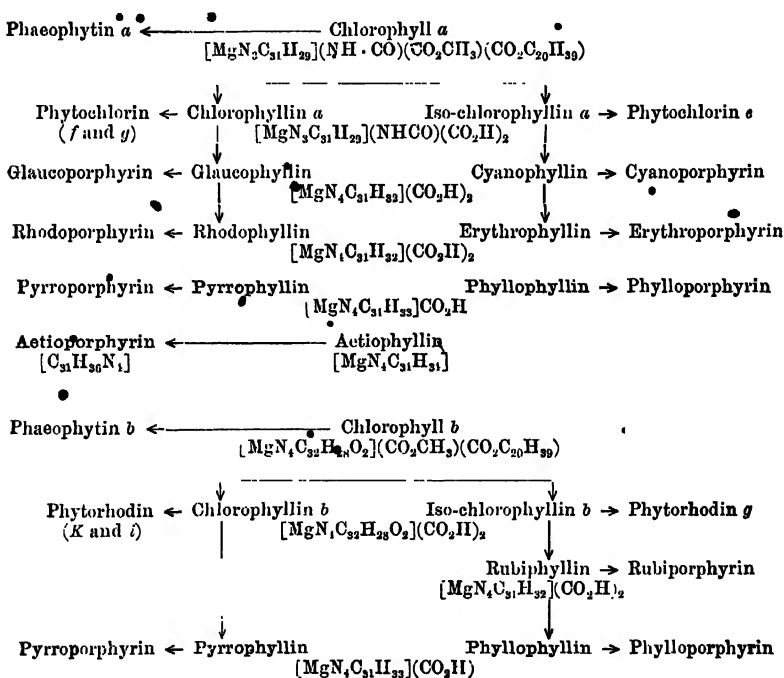


Each of the chlorophyllins is further decomposed by alkali at different temperatures into three constituents, the *phyllins* in which the lactam group is removed. Iso-chlorophyllin yields *cyanophyllin* with two carboxyl groups, giving a blue solution ; *erythrophyllin*, with

two carboxyl groups, giving a red solution ; and *phyllorphyllin*, with one carboxyl group, giving a bluish-red solution. Chlorophyllin gives a similar series, namely, *glaucophyllin*, *rhodophyllin*, and *pyrophyllin*, distinguished by similar differences in the colour of their solutions.

With soda-lime the last carboxyl group is removed and *actiophyllin* is formed. Chlorophyll *b* undergoes a similar, though by no means identical, series of transformations, but gives the same end products, namely, *pyrophyllin* and *phyllorphyllin*.

These changes may be formulated as follows, the lactam group being represented in the formulae by  $\text{NH} \cdot \text{CO}$  :



*Action of Acids on Chlorophyll.* Chlorophyll contains magnesium, which, though stable in presence of alkalis, is readily removed by mineral acids and replaced by two atoms of hydrogen, and the same change is applicable to the various derivatives. The magnesium may be restored by the action of Grignard's reagent (magnesium methyl fluoride or iodide), and in certain cases by heating with

magnesia. When chlorophyll *a* is heated with acid it yields the magnesium-free phaeophytin *a*

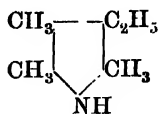


Iso-chlorophyllin gives *phytochlorin* and the various phyllins, corresponding *porphyrins* as indicated in the above table, so that the final product of both chlorophylls is *actioporphyrin*, containing neither magnesium nor oxygen.

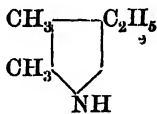
During the extraction of chlorophyll by alcohol, Willstätter and Stoll made the interesting observation that the alcoholic solution contains an active esterifying (and, at the same time, hydrolysing) enzyme, *chlorophyllase*, which replaces phytol by the alcohol used in extraction. With methyl alcohol as solvent, chlorophyll forms *methyl chlorophyllide* the crystalline neutral ester



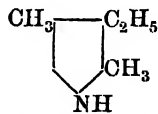
*Structure of Actiophyllin and Actioporphyrin.* As actiophyllin contains no oxygen, the magnesium is probably united to nitrogen. Phylloporphyrin, on oxidation, yields methyl ethyl maleinimide and haematinic acid, whose constitution is known and has already been discussed under haematin (p.169). The porphyrins, on reduction, give haemopyrrole, a fact first demonstrated by Nencki and Marchlewski. But haemopyrrole is not a single substance, but a mixture of three pyrrole derivatives, namely, an ethyl trimethyl and two isomeric dimethyl ethyl pyrroles of the following skeleton structure :



Phyllo-pyrrole.

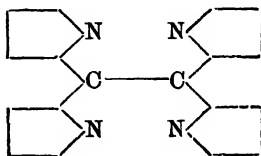


Iso-haemopyrrole.

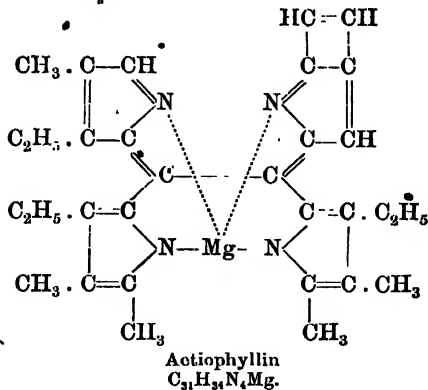
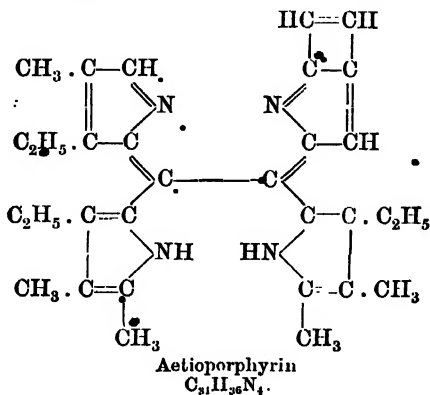


Krypto-pyrrole.

Actioporphyrin will probably be a compound of four pyrrole nuclei. Moreover, from the deficiency in hydrogen, these nuclei must be so combined and substituted by double bonds or the formation of rings that eight atoms of hydrogen less are required than if single bonds were present. These considerations lead to the skeleton framework



If to this three methyl and three ethyl groups are added, as determined by the nature of the oxidation products, and a further methyl group from that of the reduction products, the amount of hydrogen remaining will necessitate two double bonds or two carbon rings or one in each. Thus, aetiophyllin and aetioporphyrin will be most satisfactorily represented by the following formulae :



The investigation is far from having reached its final stage, nor are the views put forward here as to the composition and structure of chlorophyll and its derivatives universally admitted. There are many facts which require elucidation, and no doubt modifications in the above formulae will have to be introduced when our knowledge is more complete. As, however, the study of chlorophyll has been mainly the work of Willstätter and his collaborators, we have recorded his view on the structure of the chlorophyll

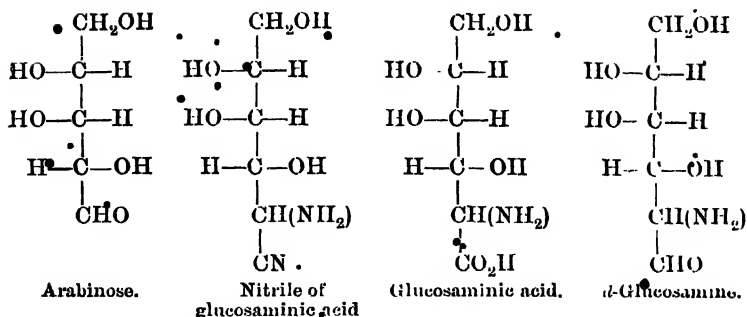
derivatives as being the best authoritative expression of the results which have been so far obtained.

*The Glucoproteins.* It has long been known that certain proteins yield, on hydrolysis, substances giving the reactions of carbohydrates, but the nature of these substances, which constitute the prosthetic groups of the glucoproteins, was obscure. The study of the carbohydrate groups of the proteins is of great physiological interest, and their investigation was stimulated by Pavy's discovery that certain typical proteins, such as egg-albumin, yield, on hydrolysis, reducing sugars. More recently it has been asserted that carbohydrate groups occur in a variety of proteins, but great caution is necessary in accepting this statement, for it is common to find that as the purification of the protein becomes more thorough, the proportion of carbohydrate obtained on hydrolysis rapidly diminishes. In many cases, however, the presence of a carbohydrate group has been demonstrated, and its nature satisfactorily determined.

The more important of the glucoproteins are known as *mucins* and *mucoids*. The mucins are viscid, tenacious substances which are produced and secreted by the epithelial cells of animals; they also form the chief constituent of the intercellular material of connective tissue. They are readily soluble in dilute alkalis, but are reprecipitated by acetic acid. The mucoids resemble the mucins in most respects, but they are not precipitated from their alkaline solutions by excess of acetic acid. F. Müller succeeded in isolating from mucine a substance which gave the carbohydrate reactions and identified it as *glucosamine*, while subsequent investigators have shown that it is somewhat widely distributed, and may constitute a large part of the glucoprotein molecule. Mucine of the trachea and mucoids from white of egg and ovarian cysts yield from 30 to 40 per cent. of glucosamine.

Glucosamine has long been known as a constituent of *chitin*, a substance which forms the exo-skeleton of many invertebrates, and its structure has recently been determined by Fischer and Leuchs,<sup>1</sup> who were able to synthesize it from arabinose. Arabinose, on treatment with ammonia and hydrocyanic acid, yields the nitrile of glucosaminic acid, and the lactone of this acid, on reduction with sodium-amalgam, gives glucosamine. Glucosamine has also been converted into glucose, and its genetic relationship with the carbohydrate established (p. 54). The relative space arrangement of the amino group is still undetermined.

<sup>1</sup> Ber., 1903, 36, 24.



Two modifications of the pentacetate and hydrochloride are known, so that glucosamine may possess a lactone structure as suggested by Irvine,<sup>1</sup> in which case it should exist in two forms.

Glucosamine may be regarded as intermediate between the carbohydrate hydrates and the amino acids, and is probably a secondary product derived from the hydrolysis of a more complicated carbohydrate group in the glucoprotein molecule. Thus Leathes<sup>2</sup> was able to isolate from ovarian mucoid a substance which resembles a disaccharose, and which, on hydrolysis, yields glucosamine and a hexose.

It has been asserted that many other prosthetic groups exist in glucoproteins, but further investigation is greatly needed. Schmiedeberg prepared from cartilage mucine a complicated substance containing sulphur which could be converted by hydrolysis successively into a substance named *chondrosin*, and into chondrosamine and glycuronic acid. Levene and La Forge<sup>3</sup> have shown that chondrosamine is isomeric with glucosamine and is probably to be represented as a *l*-allose- or *l*-altrose-amine.

**Phosphoproteins.** The phosphoproteins, as the name indicates, are proteins which contain a considerable proportion of phosphorus (0.5–1.5 per cent.). In this respect they resemble the nucleoproteins with which they were formerly classified, but they are sharply differentiated from them by the fact that, on hydrolysis, they yield neither purine bases, pyrimidine bases, nor pentoses. The most important members of this group, all of which are closely related,

<sup>1</sup> *Trans. Chem. Soc.*, 1912, 101, 1128.

<sup>2</sup> *Archiv exper. Path.*, 1899, 43, 245.

<sup>3</sup> *J. Biol. Chem.*, 1918, 15, 73, 155; 1914, 18, 124; 1915, 20, 433; 1916, 26, 143; Hudson and Dale, *J. Amer. Chem. Soc.*, 1916, 38, 1431.



are *casein*, the chief protein constituent of milk, *vitellin*, found in the yolk of eggs, and *ichthulin*, found in the eggs of fishes.

The phosphoproteins are distinctly acid substances, insoluble in water, but readily soluble in alkalis, forming definite salts.<sup>1</sup> Solutions of these salts do not coagulate on heating, but the protein is readily precipitated by acids. When casein is digested with trypsin, or with 1 per cent. caustic soda, almost the whole of the phosphorus is removed, in the latter case as an inorganic phosphate; but with trypsin, about two-thirds remains in 'organic' combination.<sup>2</sup>

The phosphoproteins yield all the ordinary protein reactions, and may be readily salted out from solutions by means of ammonium sulphate. The products of hydrolysis of casein have been very carefully studied, and, with the exception of glycine, almost all the usual amino acids have been isolated. Tyrosine and tryptophane are present in large proportion, and, in addition, Fischer and Abderhalden have isolated an acid named diaminotrioxydodecanic acid,  $C_{12}H_{24}N_2O_5$ .<sup>3</sup>

**Unclassified Proteins (Scleroproteins).** A large number of substances closely allied to the typical proteins are known, but cannot be classified under any of the above groups. It has hitherto been customary to name them 'albuminoid' substances, the term indicating their resemblance to the typical proteins. Their exclusion from the main group was due either to their physical properties or to their failure to respond to such tests as the Millon or Adamkiewicz reactions. A further study of both the true proteins and the 'albuminoids' has shown that the distinction between the groups is no longer tenable, for there is no clear line of demarcation, and no chemical tests will serve as a satisfactory basis of separation. Many of these unclassified proteins are insoluble substances, forming important constituents of the skeletal parts of living organisms, but they are not found in animal cells, blood, lymph, or similar fluids. They are all hydrolysed by acids, and many of them also by enzymes, yielding proteoses, peptones, and a variety of amino acids. The proportions of these amino acids vary considerably, which, no doubt, accounts for the physical differences between the members of this group, and the typical coagulable proteins. A few of the more important of

<sup>1</sup> The sodium salt of casein is found in commerce under the names 'plasmon' and 'nutrose'.

<sup>2</sup> Bayliss and Plimmer, *Journ. of Physiol.*, 1906, 33, 439.

<sup>3</sup> This appears to be identical with the caseinic acid of Skrap, *Ber.*, 1904, 37, 1596.

these substances are mentioned below, but for detailed information special text-books must be consulted.

*Gelatin* is obtained by the action of steam upon the insoluble protein found in bone and cartilage. On hydrolysis it yields a very large quantity of glycine (16 per cent.)<sup>1</sup> and a large amount of proline, but neither tyrosine, cystine, nor tryptophane. *Keratin* is the main constituent of hair, hoof, horns, nails, &c. It is a very insoluble substance, and is remarkable for the large amount of sulphur it contains. On hydrolysis it may yield as much as 14 per cent. of cystine.<sup>2</sup> A similar substance, known as *neurokeratin*, is found in nerve-fibres. *Elastin* is contained in the yellow elastic fibres of connective tissue and yields on hydrolysis large amounts of leucine, but scarcely more than traces of the diamino acids.<sup>3</sup> *Fibroin*, or silk fibroin, and *sericin*, or silk gelatin, are the protein constituents of crude silk. The two are separated in silk by extracting the silk-gelatin by boiling with water under pressure. The silk-fibroin which remains yields on hydrolysis more than 36 per cent. of glycine, 21 per cent. of alanine, and 10 per cent. of tyrosine, but leucine and the diamino acids are present in very small quantity, and glutamic and aspartic acids appear to be completely absent.<sup>4</sup> *Spongin* is an insoluble protein found in sponges, and contains a large proportion of iodine.

**Protein Metabolism.**<sup>5</sup> The use of protein as a food-stuff is so general in the animal kingdom that it is interesting to inquire into the chemical changes which the material undergoes and its effect in nutrition. The subject has been carefully studied for a number of years by many workers.

In the first place, Abderhalden has established conclusively that the protein in process of digestion is completely hydrolysed, so that the building up of the protein molecule from the amino-acid fragments must be carried out within the organism. Moreover, by examining the blood before and during digestion, van Slyke has been able to prove that it is in the form of amino acids that protein synthesis begins. For it is not only possible to distinguish between the more complex polypeptides and amino acids and so determine their presence or absence in the blood, but it is found that only the latter undergo metabolism and disappear, whereas the former, when

<sup>1</sup> E. Fischer, Aders, and Levene, *Zeit. physiol. Chem.*, 1902, 35, 70.

<sup>2</sup> K. A. H. Mörner, *Zeit. physiol. Chem.*, 1901, 34, 207.

<sup>3</sup> Kossel and Kutscher, *Zeit. physiol. Chem.*, 1898, 25, 551; Horbaczewski, *Zeit. physiol. Chem.*, 1898, 6, 330.

<sup>4</sup> E. Fischer and Skita, *Zeit. physiol. Chem.*, 1901, 33, 171; 1902, 35, 224.

<sup>5</sup> F. G. Hopkins, *Trans. Chem. Soc.*, 1916, 109, 629, and *Annual Report*, 1916, 13, 208.

introduced into the blood-stream, are excreted unchanged. This fact being established, the next point of interest has been to discover the amino acids which are essential or otherwise to the process of nutrition, the effects of their absence, and the special rôle which they play. This branch of investigation has been carried out chiefly by F. G. Hopkins in this country, by Osborne and Mendel and McCollum and Davis in America, and by E. Abderhalden in Germany. Hopkins fed rats with a dietary containing known amino acids in certain proportions. When the amino-acid mixture comprised the whole assembly contained in typical protein, growth was always maintained, but if hydrolysed casein (in which on hydrolysis the tryptophane is decomposed) is administered, loss of weight ensues and the animal ultimately dies. On the other hand, the addition of small quantities of tryptophane, such as occur in the products of enzyme hydrolysis, produces normal growth. The animal appears unable to synthesise the indole ring. In another case both arginine and histidine were removed, and loss of weight again took place, which was restored when these substances were replaced. On the other hand, no such effects follow in the absence of glutamic and aspartic acids, which are present to the extent of 30 per cent. in casein products. It is probable that in this case the animal dispenses with these substances because it possesses the necessary machinery for their synthesis. Tyrosine is another acid with which the animal can dispense, for gelatin, which yields none on hydrolysis, can, with the addition of tryptophane, produce growth. This result may be explained by the presence of phenylalanine, which the organism can convert into its hydroxy derivative, although the small quantity of the former has led to the view that to make up the deficiency the benzene ring is synthesised. Although, as previously stated, the absence of histidine and arginine cause decrease in weight, if either is present the curve of body-weight rises. That the two should be apparently interchangeable seems not improbable from a comparison of their closely related structure. Moreover, it seems from Hopkins' experiments that these two protein products play a special part in purine metabolism.

The work of Osborne and his collaborators<sup>1</sup> is chiefly concerned with the nutritive value of different pure vegetable proteins of known amino-acid content whereby a similar selective effect has been demonstrated. Like Hopkins, he finds that proteins deficient in trypto-

<sup>1</sup> *Zeit. physiol. Chem.*, 1912, 80, 307; *Journ. Biol. Chem.*, 1912, 13, 233, and other papers.

phang (phaseolin, zein, and gelatin) diminish the animal weight, whereas most of the other animal and vegetable proteins, especially lactalbumin, produce an increase, and concludes that the animal organism is incapable of synthesising cyclic amino acids, for which it is dependent on vegetable proteins. In this he includes tyrosine and phenylalanine; but subsequent researches seem to point to the synthetic power of the organism being capable of elaborating both compounds (see above). Seeing that such very different proteins as gliadin, edestin, and casein are deficient in either lysine, glycine, phosphoproteins, or purines, the activities of the animal organism must be utilized in the synthesis of these substances.

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*The Chemical Constitution of the Proteins*, Part I. 2nd ed., and Part II, by R. H. Aders Plimmer. Monographs on Biochemistry. Longmans, 1912.  
*The Vegetable Proteins*, by T. B. Osborne. Monographs on Biochemistry. Longmans, 1909. •  
*The General Characters of the Proteins*, by S. B. Schryver. Monographs on Biochemistry. Longmans, 1909.  
 \*Special articles in Asher and Spiro's *Ergebnisse der Physiologie*, including *Ueber Bau und Gruppierung der Eiweisskörper*, by F. Hofmeister. The latter paper includes an excellent bibliography.

## CHAPTER V

### / THE TERPENES AND CAMPHORS

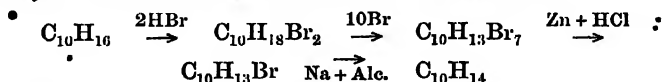
**The Terpenes.** The terpenes are hydrocarbons of the formula  $C_{10}H_{16}$ , which are found widely distributed and in considerable quantities in the *essential oils* of plants. They occur in different parts of the plant, sometimes singly, sometimes two or more together, and very frequently associated with *sesquiterpenes*  $C_{15}H_{24}$ , and with closely allied compounds containing oxygen, such as *thymol* and *carvone*,  $C_{10}H_{14}O$ , *cineol*, *fenchone*, *thujone*, and *camphor*,  $C_{10}H_{16}O$ , *terpineol* and *borneol*,  $C_{10}H_{18}O$ , *menthol*,  $C_{10}H_{20}O$ , &c. A more complete account of these oils is given in the section on natural and artificial perfumes (p. 259).

The terpenes have been the subject of careful and elaborate investigation during the last twenty years, chiefly at the hands of Prof. Wallach, who has devoted himself with signal success to unravelling the tangled web of facts scattered through the literature of the subject, and to developing methods of identifying the members of this large family of isomeric compounds. He has, moreover, traced the relations which subsist between them, and brought our knowledge to a point at which the difficult problems of structure may be considered to be within measurable distance of solution.

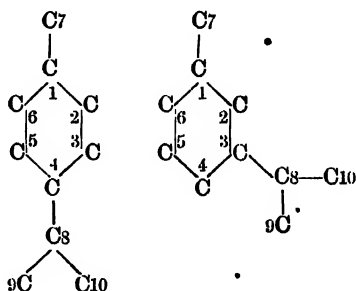
**Properties of the Terpenes.** More than a dozen distinct compounds of the formula  $C_{10}H_{16}$  are at present known; among which are *d*- and *l*-*limonene* and the racemic form of *dipentene*, *terpinolene*,  $\alpha$ - and  $\beta$ -*terpinene* and  $\alpha$ - and  $\beta$ -*phellandrene*, *d*-*sylvestrene*, and its inactive form, *carvestrene*, *d*-*l*- $\alpha$ -*pinene*,  $\beta$ -*pinene*, *d*-*l*-*camphene*, *bornylene*, *fenchene*, *sabinene*, *thujene*, &c. With the exception of *terpinolene*, *fenchene*, *bornylene*, *carvestrene*, and *thujene*, they are found in nature. *Camphene* and *bornylene* are solids at the ordinary temperatures, but the remainder are liquids which distil unchanged at temperatures varying from  $155^{\circ}$  to  $185^{\circ}$ . They have a high refractive index (1.46–1.47) and a low specific gravity (0.84–0.86).

They appear to be all cyclic compounds standing midway as to properties between benzene hydrocarbons and the olefines. On the one hand they form additive compounds with the halogens, halide acids, nitrosyl chloride, nitrogen trioxide, and tetroxide, &c., and, on

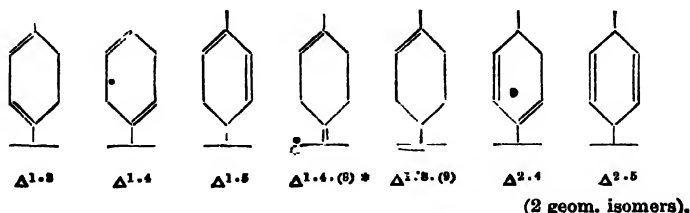
the other, they are convertible into *p*-cymene, and in a few cases into *m*-cymene. By exhaustive bromination and subsequent reduction with zinc and hydrochloric acid, and then with sodium and alcohol, according to the method of Baeyer and Villiger,<sup>1</sup> limonene has been converted into *p*-cymene and sylvestrene into *m*-cymene.



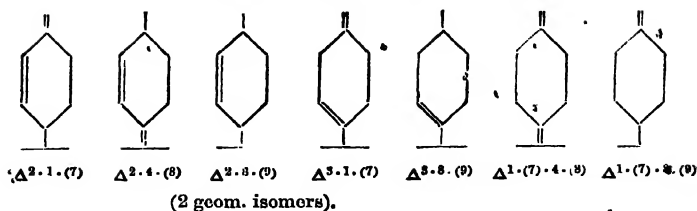
**Classification of the Terpenes.** In accordance with our present knowledge of their structure, which is partly derived from their optical characters, activity, molecular refraction, and magnetic rotation, but mainly from their chemical behaviour, the terpenes are separated into a mono-cyclic and a bi-cyclic group. The first group may be regarded as dihydro-derivatives of *p*- and *m*-cymene,  $\text{C}_{10}\text{H}_{14}$ , or, what is synonymous, as unsaturated derivatives of hexahydro-cymene or menthane,  $\text{C}_{10}\text{H}_{20}$ . For purposes of nomenclature the latter view is adopted, and the members of the group are described as *menthadienes*, that is, menthane with two double linkages. The position of these linkages is indicated in the usual way (Part II, p. 397), by the use of  $\Delta$  in conjunction with the numbered position of the ten carbon atoms.



It will be at once perceived how very large a number of possible menthadienes can be derived from these two skeleton structures. To take the case of *p*-menthadiene, there are fourteen isomers which may be represented for brevity by the following formulae.

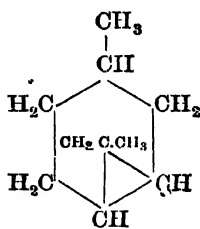
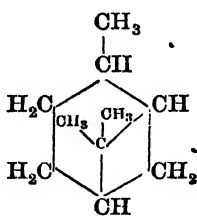


<sup>1</sup> Ber., 1898, 31, 1401, 2067.

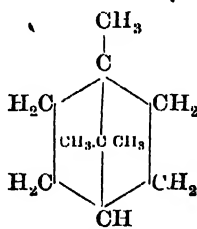


\* The bracketed numbers indicate the second carbon attachment of the double bond outside the nucleus.

Compounds of this class are naturally able to combine with two molecules of halogen, halide acid, &c. Thus, limonene forms a dihydrobromide,  $C_{10}H_{16} \cdot 2HBr$ , with hydrogen bromide, and a tetrabromide,  $C_{10}H_{16}Br_4$ , with bromine. The second group of bi-cyclic compounds can, on the other hand, only unite with one molecule of halogen and halide acid, and, for this and other reasons to be presently discussed, they are assumed to have a bridged-ring structure. Three bridged rings are now recognized as the basis of the second group, and the corresponding saturated hydrocarbons,  $C_{10}H_{18}$ , have been prepared and are known respectively as *carane*,<sup>1</sup> *pinane*,<sup>2</sup> and *camphane*.<sup>3</sup>

Carane.<sup>4</sup>

Pinane.



Camphane.

It follows that the terpenes derived from them will contain one double bond only, and may be described as *carene*, *pinene*, and *camphene*. The position of the double bond will be discussed in the sequel. It is clear, however, that the opportunities for isomerism are more restricted than in the case of the menthadienes. With

<sup>1</sup> Sommler and Feldstein, *Ber.*, 1914, 47, 384.

<sup>2</sup> Sabatier and Senderens, *Compt. rend.*, 1901, 132, 1254.

<sup>3</sup> Aschan, *Annalen*, 1901, 316, 229; Henderson and Pollock, *Trans. Chem. Soc.*, 1910, 91, 1620.

<sup>4</sup> For brevity the following symbols will be occasionally used to indicate these three types of bridged rings.



this brief account of the system of classification and nomenclature, we will pass at once to the study of the properties and structure of the individual terpenes, beginning with the simpler class of menthadienes.

## • MONO-CYCLIC TERPENES (MENTHADIENES).

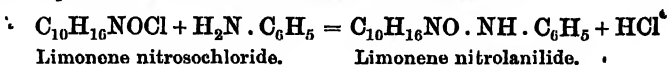
**d-l-Limonene (Dipentene).** *D*- and *l*-limonene and the racemic form of dipentene are among the most widely distributed constituents of essential oils. They possess the characteristic smell of lemon. The *d*-compound is found in oil of lemons, neroli (from orange flowers), orange (from the rind), bergamot, limette, caraway, dill, &c. • • •

The *l*-compound is less common and is a constituent of pine-needle oil from *Abies alba*, Russian and American spearmint oil, American peppermint oil, &c., whilst the inactive dipentene is found in pine-needle oil, citronella oil, oil of cubebs, &c. It is also formed by mixing the two active limonenes, or by racemising them singly at a high temperature. Many other terpenes yield dipentene on continued heating, a fact which may explain its presence in the products of the dry distillation of rosin and caoutchouc. Another interesting source of dipentene is pinene, the chief constituent of ordinary American turpentine oil, from which it may be obtained directly by the action of alcoholic sulphuric acid or, indirectly, by the aid of moist hydrogen chloride, which transforms pinene into the dihydrochloride of dipentene,  $C_{10}H_{16} \cdot 2HCl$ . The explanation of these changes will be discussed under pinene (p. 211). Dipentene can also be obtained from the closely allied substances *terpinol* (m. p. 35°) and *cincol*, which are described later. Dipentene is usually separated and purified by conversion into the crystalline dihydrochloride (by passing gaseous hydrogen chloride into the liquid), from which the terpene is regenerated by boiling the hydrochloride with anhydrous sodium acetate dissolved in acetic acid. This method cannot be applied to the limonenes, for they are racemised in the process. Pure limonene is purified by conversion into the crystalline tetrabromide,  $C_{10}H_{16}Br_4$ , from which it is liberated by reduction with zinc dust and alcohol. *l*-Limonene can also be obtained from active dihydrocarveol by the xanthic ester method of Tschugaeff, which is described later (p. 227). Highly characteristic of dipentene and the limonenes are the compounds with nitrosyl chloride.<sup>1</sup> They are crystalline substances of the formula  $C_{10}H_{16}NOCl$ , which are best obtained by adding amyl nitrite dissolved in glacial acetic acid to the terpene and acidifying with strong hydrochloric acid. As the addition of

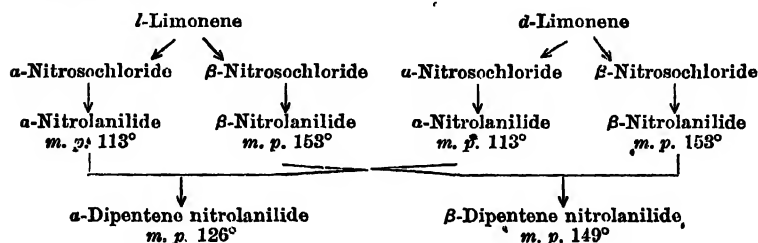
<sup>1</sup> Tilden and Shennstone, *Trans. Chem. Soc.*, 1874, 13, 514.



nitrosyl chloride introduces a new asymmetric carbon into the molecule, as will presently appear, it is scarcely surprising that each of the limonenes, as well as dipentene, should yield two products,  $\alpha$ - and  $\beta$ -nitrosochlorides. By the action of aniline on each of the six compounds a different nitrolanilide is obtained.



The  $\alpha$ - and  $\beta$ -*d*-nitrosochlorides will be enantiomorphous with the  $\alpha$ - and  $\beta$ -*l*-nitrosochlorides, so that by mixing equivalents of *d*- and *l*- $\alpha$ -nitrosochloride and *d*- and *l*- $\beta$ -nitrosochloride, the two dipentene compounds are obtained. The same is of course true, of the nitrolanilides.



In addition to a nitrosochloride compound, dipentene forms a nitrosate,  $\text{C}_{10}\text{H}_{16}\text{NO} \cdot \text{ONO}_2$ , by the combined action of nitric acid, amylnitrite, and acetic acid.

**Structure of Limonene (Dipentene).** In attempting to ascertain the constitution of these compounds it will be necessary in the first place to describe certain other substances to which they are related.

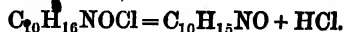
The chief interest centres round the structure of carvone and terpineol. Both compounds are found in nature accompanying the terpenes, and both are closely related to dipentene, the limonenes, and terpinolene.

*Carvone*,  $\text{C}_{10}\text{H}_{14}\text{O}$ , formerly known as carvol, is found as the *d*-form in dill and caraway oil, and as the *l*-modification in spearmint and kuromoji oil, and possesses the characteristic smell of caraway oil. It is isolated and purified by conversion into a curious crystalline compound which it forms with hydrogen sulphide,  $(\text{C}_{10}\text{H}_{14}\text{O})_2\text{H}_2\text{S}$ .

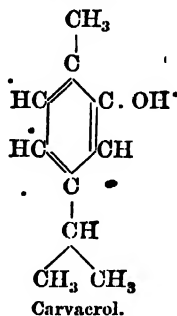
It is a ketone and forms a carvoxime,  $\text{C}_{10}\text{H}_{14}:\text{NOH}$ , which Goldschmidt and Zürrer<sup>1</sup> found to be identical with the nitroso-

<sup>1</sup> *Ber.*, 1885, 18, 2220.

limonene obtained by Tilden and Shenstone<sup>1</sup> by the action of alcoholic potash on the nitrosochloride,



Now carvone is easily converted by heating with phosphoric acid and with certain other reagents into the isomeric *carvacrol*, the structure of which is known. It is a hydroxy *p*-cymene of the formula:



We may therefore assume that in this reaction an isomeric change occurs in carvone, whereby a hydrogen atom from some other part of the molecule attaches itself to the oxygen of the ketone group situated in the nucleus. If this is the case carvone will contain two double bonds, an assumption which is borne out by its behaviour. Carvone behaves like an  $\alpha\beta$  unsaturated ketone, for with a mild reducing agent, such as zinc dust and alcohol, it takes up a molecule of hydrogen and passes into *dihydrocarvone*; with a stronger reducing agent, such as sodium and alcohol, the ketone group is also reduced and the secondary alcohol, *dihydrocarveol*, is formed, from which dihydrocarvone may be regenerated by oxidation. Both dihydrocarveol and dihydrocarvone are still unsaturated, for they combine with a molecule of hydrogen bromide, and further reduction converts them into *tetrahydrocarveol* (carvomenthol), which is a saturated secondary alcohol, yielding the ketone, *tetrahydrocarvone*, on oxidation. Having established the unsaturated nature of carvone, the next problem is to ascertain the positions of the two pairs of double bonds, for these have a special interest, seeing that they are probably common to both carvone and limonene. The problem has been approached by attempting to locate the position of each double bond separately, dihydrocarveol, which only contains one double bond, being first selected for the purpose.

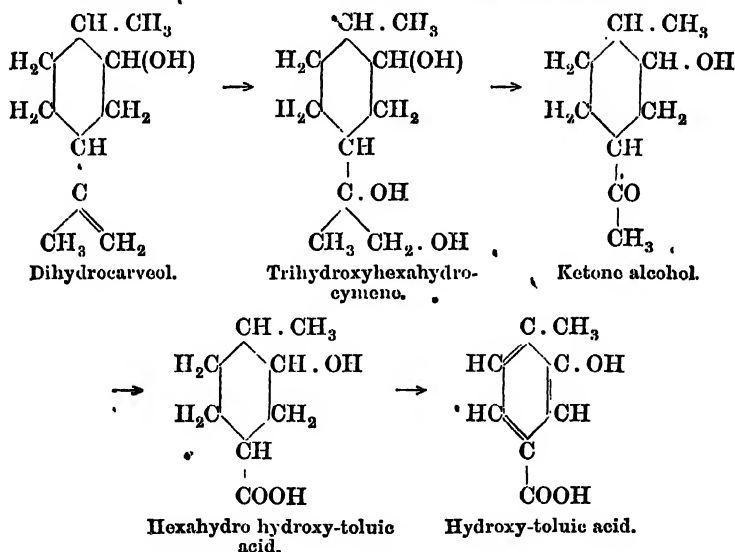
It is well known that unsaturated compounds are readily oxidised by permanganate solution, the first stage being the addition of two

<sup>1</sup> *Trans. Chem. Soc.*, 1877, 31, 554.

hydroxyl groups to the doubly linked carbon atoms, which is then followed by the rupture of the compound at the original double bond.

The action of permanganate solution upon dihydrocarveol has been carefully studied by Wallach,<sup>1</sup> and also by Tiemann and Semmler.<sup>2</sup> Two hydroxyls are first introduced and a trihydroxyhexahydrocymene (menthanetriol) is obtained. Chromic acid converts the latter into a ketone alcohol of the formula  $C_9H_{16}O_2$ , which on oxidation with sodium hypobromite yields an acid,  $C_7H_{12}(OH).COOH$ , and this in turn, on being oxidised with bromine, gives *m*-hydroxy-*p*-toluic acid,  $C_7H_6(OH).COOH$ .

The simple explanation of these changes is based by Tiemann on the assumption of a double bond in the isopropyl side-chain.



If this view is correct the double bond in carvone and limonene is established. The position of the second double bond has been ascertained by reference to the structure of terpineol (m. p.  $35^\circ$ ), which in turn is derived from that of terpin  $C_{10}H_{18}(OH)_2$ .

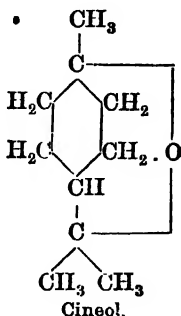
*Terpin* is the alcohol corresponding to dipentene dihydrobromide, for it can be obtained from the latter by the action of silver acetate and subsequent hydrolysis, or it may be converted by the reverse process into dipentene dihydrobromide with hydrogen bromide. Terpin is usually prepared by the action of strong nitric acid on an alcoholic solution of turpentine from which it crystallizes as the

<sup>1</sup> *Annalen*, 1893, 275, 110.

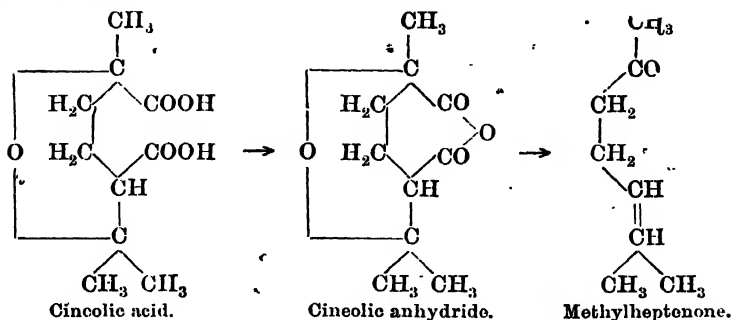
<sup>2</sup> *Ber.*, 1895, 28, 2141.

hydrate  $C_{10}H_{18}(OH)_2 \cdot H_2O$ , and the method is interesting as it affords a connecting link between the two terpenes, dipentene and pinene. Terpin exists in two stereoisomeric modifications as *cis* and *trans* terpin (Part II, p. 243). The *trans* form, which is the more soluble, is derived from dipentene dihydrobromide, whereas the *cis* modification is prepared from turpentine or from terpinene (see below) by shaking with dilute sulphuric acid. It is the latter which forms the hydrate. Although neither form of terpin has so far been found in nature, both are readily converted by dehydrating agents into two well-known natural products which accompany the terpenes in many of the essential oils, namely, cineol and terpineol (m. p.  $35^\circ$ ) (which are isomeric compounds of the formula  $C_{10}H_{18}O$ ), as well as terpinene, terpinolene, and dipentene, all of which are closely related.

*Cineol* has no real connection with the problem under discussion, but by virtue of its interest as a natural product it merits a short description. As already stated, it is a common constituent of many essential oils and is specially abundant in eucalyptus, cajeput, and wormseed oil (*olcum cinæ*). It is a liquid with a characteristic camphor-like smell and boils at  $177^\circ$ . As it possesses neither alcoholic nor ketonic properties and is closely related to terpin, it is regarded as an inner ether of that compound. Its structure therefore depends upon that of terpin, which is the subject of the present inquiry, and may be provisionally represented as follows:

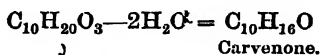
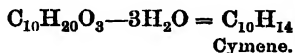


The correctness of this formula is determined by its products of oxidation, whereby it yields a dibasic acid, cineolic acid. The latter gives an anhydride with acetic anhydride, which on heating is converted into methyl heptenone of known structure.



Cineol combines with hydrogen bromide forming *cis* "dipentene dihydrobromide."

*α*-Terpineol ( $\Delta^1$  Menthon 8-ol) (m. p. 35°) is one of a numerous class of unsaturated alcohols of the formula  $C_{10}H_{17}OH$ , known generally as terpineols or menthonols. It occurs in nature in active and inactive forms and has an odour of lilac. The dextro form is found in lovage oil, cardamom, and marjoram oil; the laevo form in niaouli oil, and the inactive form in cajeput oil. It is conveniently prepared by shaking terpin hydrate with dilute sulphuric acid and separating the solid terpineol from the liquid products by freezing. It contains one tertiary alcohol group and one double bond, for it forms a phenylurethane with phenyl carbimide  $C_{10}H_{17} \cdot O \cdot CONHC_6H_5$ , and unites with one molecule of nitrosylchloride and of bromine. The hydroxyl group will probably occupy the same position as one of the terpin hydroxyls and therefore the same position as one of the halogen atoms in dipentene dihydrobromide. As in the case of dihydrocarveol, the action of permanganate has led to very interesting results, for which we are indebted to Wallach.<sup>1</sup> The first product is a nearly quantitative yield of a trihydroxyhexahydrocymene (menthane triol), which is, however, not identical with that from dihydrocarveol (see p. 190), for on warming with dilute sulphuric acid it is decomposed into cymene and carvenone, while the other is not.

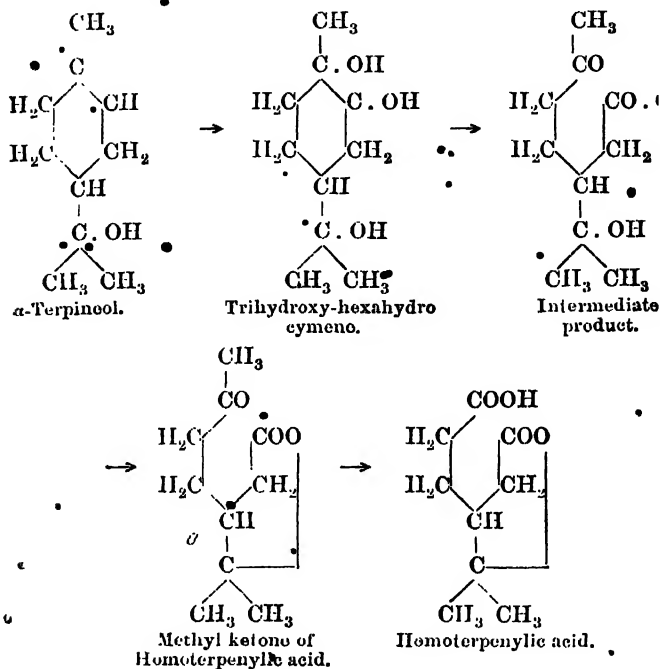


These changes will be discussed later (see p. 205). On further oxidation of the trihydroxy compound with chromic acid, it is converted successively into homoterpenylic acid and into terpenylic acid, the structure of which is known through Lawrence's<sup>2</sup> and

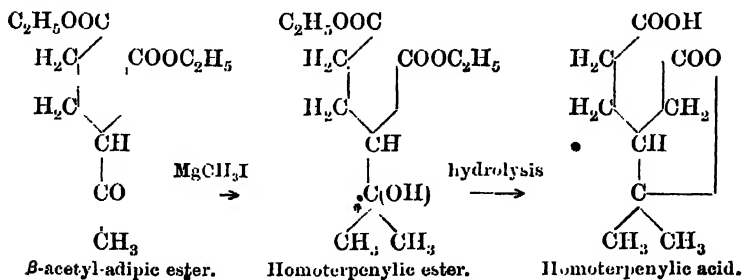
<sup>1</sup> *Annalen*, 1893, 277, 110.

<sup>2</sup> *Trans. Chem. Soc.*, 1899, 75, 527.

Simonsen's synthesis (see below). Adopting Wagner's formula for terpineol,<sup>1</sup> the results are explained by Wallach in a very simple fashion, as follows: .



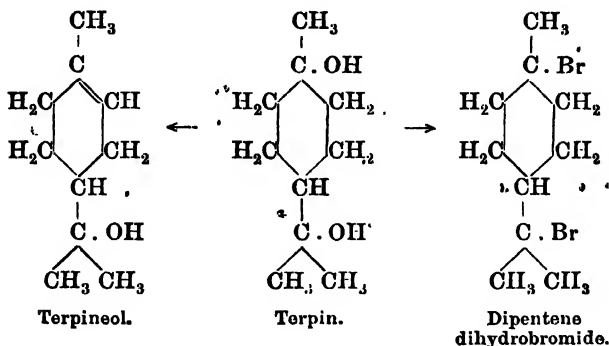
Simonsen<sup>2</sup> obtained homoterpenylic, terpenylic, and terebic acids by the action of the Grignard reagent on  $\beta$ -acetyl-adipic, -glutaric, and -succinic esters. The process in each case is similar, so that one example will suffice.



<sup>1</sup> Ber., 1890, 23, 2307.

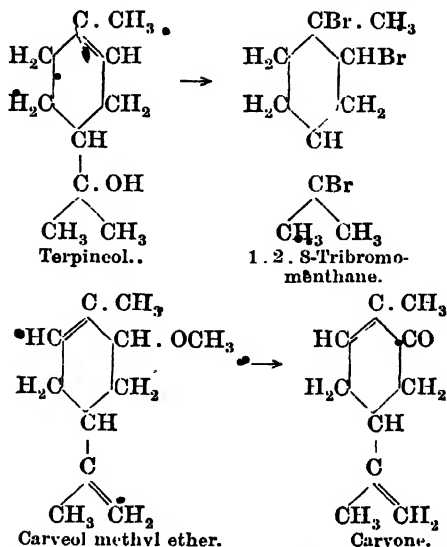
<sup>2</sup> Trans. Chem. Soc., 1907, 91, 184.

It follows that as terpin is a bi-tertiary alcohol, its relation to terpineol on the one hand and dipentene dihydrobromide on the other must be represented as follows:

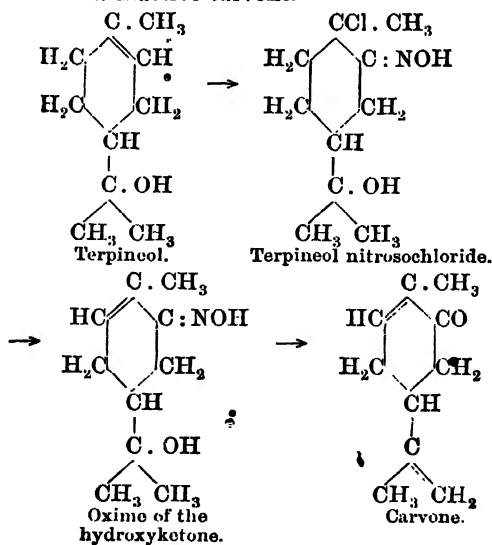


The existence of a *cis* and *trans* terpin (Part II, p. 262), and of corresponding dihydrobromides of dipentene, accords well with these formulae; but the structure of dipentene itself is still ambiguous, as the two molecules of hydrogen bromide may be removed from the hydrobromide in various ways. Now it has been shown that, as the oxime of carvone is identical with nitrosolimonene, the double bonds of carvone are probably identical in position with those of limonene. The position of one of these double bonds is fixed beyond question by the structure of dihydrocarveol; the other is probably present in terpineol. The final evidence must rest on the relation of terpineol to carvone, and this has been furnished by converting terpineol into carvone, and vice versa. Wallach<sup>1</sup> was the first to convert terpineol into carvone by the following rather lengthy process: terpineol forms a dibromide  $\text{C}_{10}\text{H}_{17}\text{Br}_2 \cdot \text{OH}$ , and this on standing with hydrobromic acid loses hydroxyl, which is replaced by bromine forming a tribromide (1. 2. 8-tribromomenthane) of the formula  $\text{C}_{10}\text{H}_{17}\text{Br}_3$ . Warmed with sodium methylate, carveol methyl ether is produced,  $\text{C}_{10}\text{H}_{17} \cdot \text{OCH}_3$ , two molecules of hydrogen bromide being removed and the third bromine atom replaced by methoxyl. This compound gives carvone on oxidation:

<sup>1</sup> *Annalen*. 1894. 281. 140.



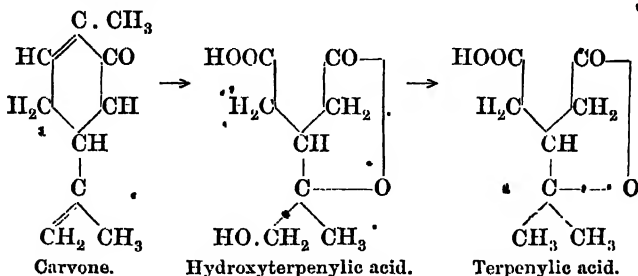
A simpler method, also devised by Wallach for effecting the same result is to convert terpinol into the nitrosochloride and by removing hydrogen chloride to obtain the corresponding oxime, and finally, by boiling with acids, inactive carvone.<sup>1</sup>



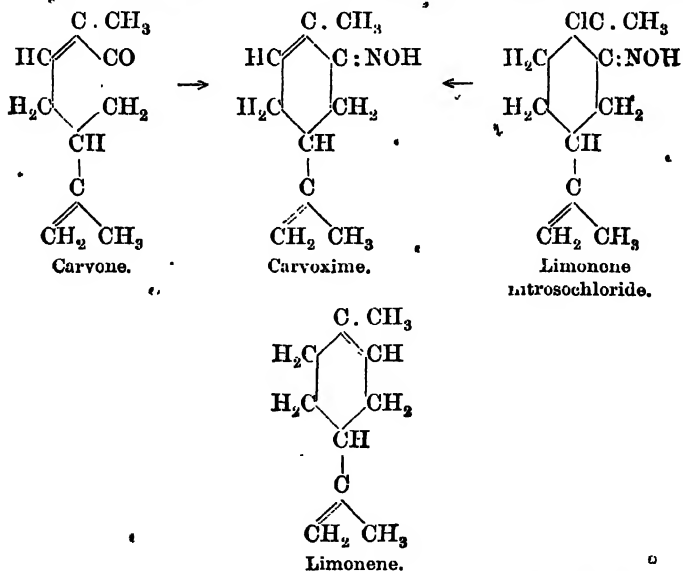
<sup>1</sup> *Annalen*, 1893, 277, 120.



On closer observation it will be seen that neither method makes it quite clear that the double bond lies in the nucleus, and not between the methyl group and the nucleus. Even the oxidation of carvone to terpenylic acid, which has also been effected,<sup>1</sup> does not remove such a possibility.



Assuming, however, the structure of carvone to be that given above, the formula for limonene and dipentene naturally follows.

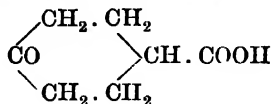


If any real doubt existed as to the correctness of the formula, it has now been to a great extent removed by the synthesis of terpin, terpineol, and dipentene, by W. H. Perkin, jun.<sup>2</sup>

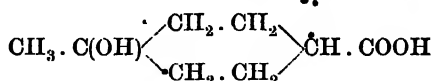
<sup>1</sup> Best, *Ber.*, 1894, 27, 1218; Wallach, *Ber.*, 1894, 27, 1495.

<sup>2</sup> *Trans. Chem. Soc.*, 1904, 85, 654.

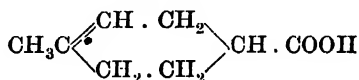
Perkin's synthesis is effected in the following manner. The starting-point is  $\delta$ -ketohexahydrobenzoic acid.<sup>1</sup>



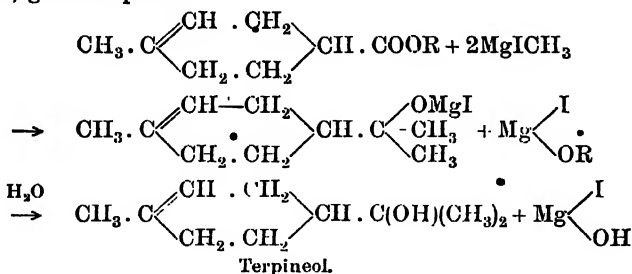
Its ester reacts readily with magnesium methyl iodide, and the product on hydrolysis yields  $\delta$ -hydroxyhexahydro-*p*-toluic acid.



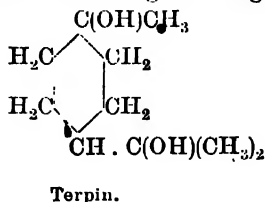
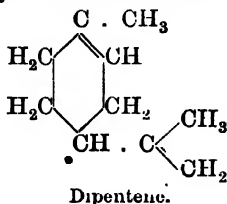
This hydroxy acid dissolves readily in fuming hydrobromic acid, and the solution soon deposits crystals of  $\delta$ -bromohexahydro-*p*-toluic acid, from which, on treatment with weak alkalis or pyridine,  $\Delta^8$  tetrahydro-*p*-toluic acid is obtained.



The ester of this acid, when acted on with magnesium methyl iodide, gives terpineol.<sup>2</sup>



The latter is transformed on the one hand into dipentene by the action of potassium hydrogen sulphate, and on the other into terpin hydrate by shaking with dilute sulphuric acid, or directly from the original cyclohexanone ester with excess of the Grignard reagent.

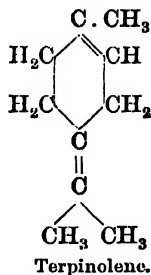
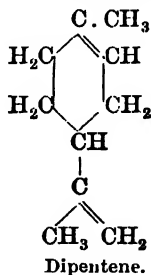
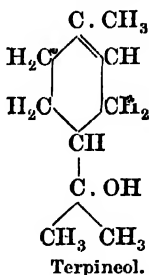


<sup>1</sup> *Trans. Chem. Soc.*, 1904, 85, 138, 416; 1906, 89, 1640; 1907, 91, 372.

In order to obtain the active terpineol and terpene, the compound is resolved at the tetrahydrotoluic acid stage by fractional crystallization of the strychnine and brucine salts. In this way *d*- and *l*-terpineol were obtained, but the limonenes, like most of the artificial terpenes, racemise so readily that they could not be prepared pure in this way.

The structure of dipentene being thus satisfactorily settled, its formation from terpin, terpineol, and cineol by dehydrating agents is readily understood without further explanation. But dipentene is not the only product of these reactions, for two other terpenes make their appearance at the same time, namely, terpinolene and terpinene.

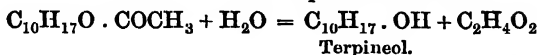
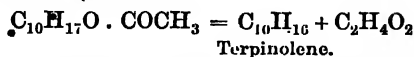
**Terpinolene.** Terpinolene is one of the artificial and inactive terpenes, which was found by Wallach among the products obtained by the inversion of pinene by means of alcoholic sulphuric acid. It can also be prepared, as already stated, from terpin, terpineol, or cineol, by boiling with dilute sulphuric or phosphoric acid. Terpinolene is readily converted into terpinene (see p. 199), and, where sulphuric acid is used, cymene is also produced. The best method for obtaining it is either by the action of oxalic acid on terpineol (m. p. 35°) or by that of dilute acids on  $\gamma$ -terpineol (p. 203). Since the elements of water may be eliminated from the two adjacent carbon atoms of terpineol in two ways, one of which yields dipentene, the other derivative will probably represent terpinolene.



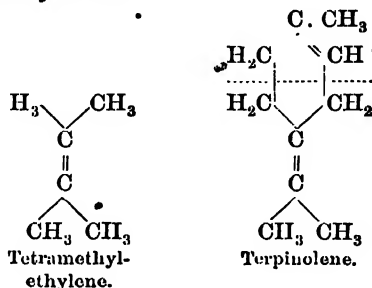
This line of argument would not in itself carry much weight, a similar reaction yielding terpinene, had not independent evidence been forthcoming in support of the above constitution. Terpinolene has been obtained by Baeyer<sup>1</sup> from dipentene tribromide,  $\text{C}_{10}\text{H}_{17}\text{Br}_3$ , the latter being obtained by brominating dipentene dihydrobromide. If the tribromide is treated with zinc dust and acetic acid, two atoms of bromine are removed and the third replaced by hydroxy-acetyl, forming terpineol acetate,  $\text{C}_{10}\text{H}_{17}\text{O} \cdot \text{COCH}_3$ , and this on distillation

<sup>1</sup> Ber., 1894. 27. 443. 815.

with quinoline yields acetic acid and terpinolene, or, on hydrolysis, gives a new terpineol (*m. p.* 70°).

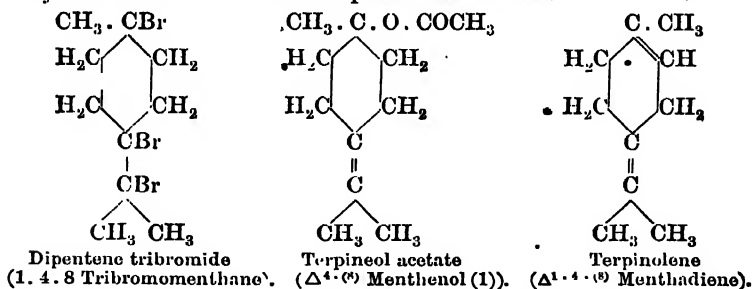


The evidence upon which the structure of terpineol acetate rests is the formation of a blue crystalline nitroschloride, which is indistinguishable in appearance from the nitroschloride of tetramethylethylene prepared by Thiele.<sup>1</sup>



Baeyer concluded that both compounds possess a similar structure.

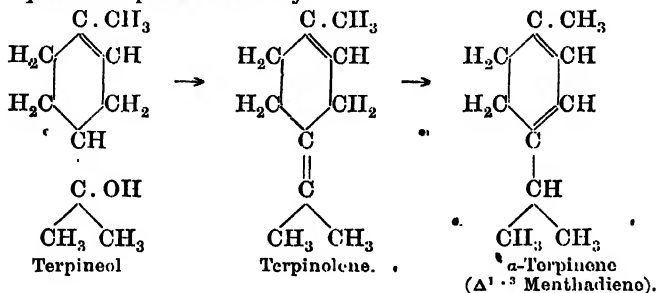
The various steps in the preparation of terpinolene from dipentene dihydrobromide will then be expressed as follows:



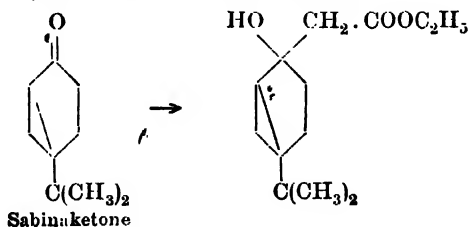
**Terpinenes.** α-Terpinene is formed, as already stated, from terpin hydrate, terpineol, and cineol, and substances such as pinene and dipentene, which are easily converted into terpin hydrate by means of sulphuric acid; but it is most readily obtained by shaking turpentine oil (pinene) with small successive quantities of strong sulphuric acid, and from sabinene (*p.* 203). So far its occurrence in nature seems very restricted, for it has been found in few of the essential oils. Its presence in cardamom oil was first noticed by Weber, who identified it by means of the crystalline nitrosite,  $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3$ , which

it gives with nitrous acid, and which is characteristic both of terpinene and phellandrene. It forms crystalline compounds with two molecules of halide acids which determine the presence of two double bonds, and furthermore gives *aa'*-methyl isopropyl *aa'*-dihydroxyadipic acid on oxidation. This, together with its synthesis from sabinaketone, described below, leaves no doubt that it contains two conjugated double bonds in the nucleus, and is a  $\Delta^{1,3}$  menthadiene. According to Wallach,<sup>1</sup> it is generally accompanied by  $\gamma$ -terpinene or  $\Delta^{1,4}$  menthadiene.

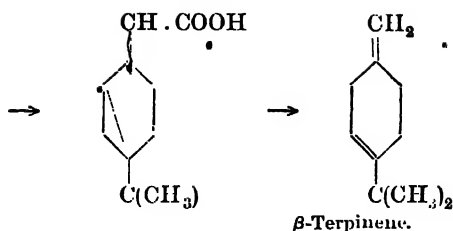
It is probable that the formation of terpinene from terpinolene and terpineol may indicate a shifting of the double bond of terpinolene from the side-chain to the nucleus, and such a structure would also explain its optical inactivity.



In addition to the above, there exists a  $\beta$ -terpinene ( $\Delta^{1(7)}$  menthadiene) which was obtained synthetically by Wallach in 1907 from sabinaketone by a method which has been frequently utilized by him for the purpose of replacing the oxygen of a cyclic ketone group by the unsaturated side-chain  $=\text{CH}_2$  in the following way: The ketone is condensed with bromoacetic ester in presence of zinc according to Reformatsky's reaction (Part I, p. 217). The ester is then hydrolysed and heated with acetic anhydride, which converts it into an unsaturated acid, and the latter, on heating alone, loses carbon dioxide and water and gives the unsaturated side-chain, whilst the bridged ring becomes an ethenoid linkage.

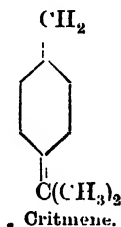


<sup>1</sup> *Annalen*, 1908, 362, 235.



$\beta$ -Terpinene combines only slowly with nitrous acid to form a nitrosite; it gives an insoluble crystalline tetrabromide, not given by  $\alpha$ -terpinene, and oxidises readily in contact with air.

A fourth terpinene termed critmene from *crithmum maritimum* has been examined by Francesconi and Sernagiotto,<sup>1</sup> who have given it the following formula:



**Phellandrenes.**  $\alpha$ -Phellandrene, like  $\alpha$ -terpinene, forms a crystalline nitrosite, and in this form it was first isolated by Cahours<sup>2</sup> in 1842. It has since been found in two active modifications, the *d*-compound occurring in bitter- and water-fennel oil (*Phellandrium aquaticum*) and elemi oil, and the *l*-compound in Australian eucalyptus oil and pine-needle oil. It is very sensitive to acids, and with alcoholic sulphuric acid is converted into terpinene.

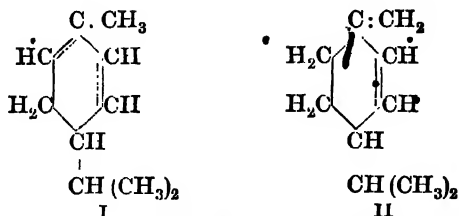
In the year 1904 Wallach<sup>3</sup> published a very complete investigation on the nature of phellandrene, and pointed out that in addition to ordinary or  $\alpha$ -phellandrene a second or  $\beta$ -phellandrene is present in water-fennel oil. The first was shown to have the structure of  $\Delta^{2,5}$  dihydrocymene (I) by the conversion of nitrophellandrene into active menthenone (carvotanacetone), whilst  $\beta$ -phellandrene, which formed the subject of a subsequent investigation by Wallach,<sup>4</sup> was shown to possess the formula II by synthesis from  $\Delta^2$  isopropylcyclohexenone (p. 229) after the manner of  $\beta$ -terpinene:

<sup>1</sup> *Atti R. Accad. Lincei*, 1913, 22, 382.

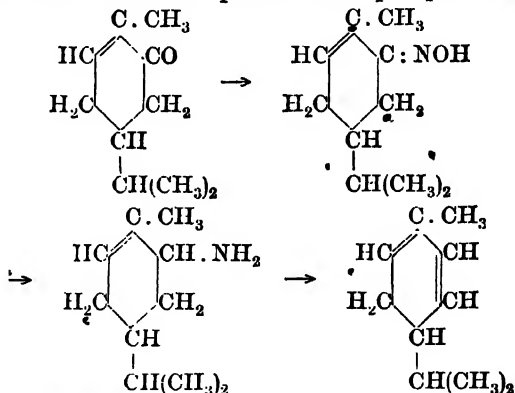
<sup>3</sup> *Annalen*, 1904, 336, 9.

<sup>2</sup> *Annalen*, 1842, 41, 74.

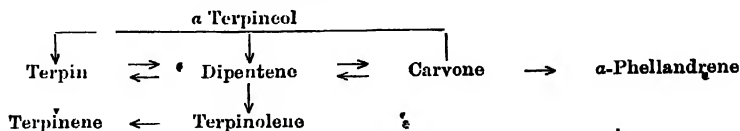
<sup>4</sup> *Annalen*, 1905, 340, 1.



Harries and Johnson<sup>1</sup> confirmed the formula given to  $\alpha$ -phellandrene by the reverse process to that employed by Wallach, namely, by converting *d*-menthenone into phellandrene as follows: menthenone is first treated with phosphorus pentachloride, and the resulting chloride converted into chlorophellandrene by boiling with quinoline. The chlorophellandrene, on reduction with zinc dust in methyl alcohol, yields  $\alpha$ -phellandrene. Another and better method is to reduce menthenoneoxime to  $\Delta^6$  menthenamine, and to distil the product under diminished pressure with phosphoric acid.



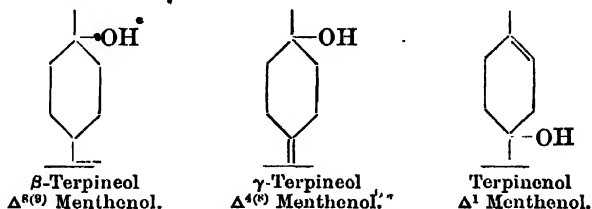
**The Terpeneols (Menthenols).** It will be observed from the facts stated in the foregoing sections that  $\alpha$ -terpineol has been brought into relationship with the following terpenes :



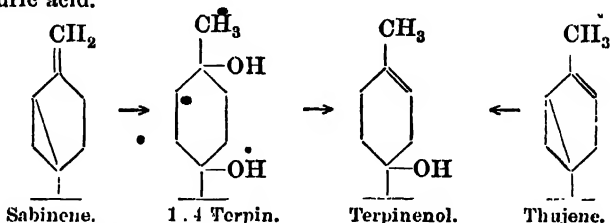
In addition to  $\alpha$ -terpinol, there exist  $\beta$ - and  $\gamma$ -terpineol, terpinenol, and numerous menthenols obtained synthetically by Perkin and others, the preparation of which are described under synthetic terpenes (p. 206).

<sup>1</sup> Ber., 1905, 38, 1332.

$\beta$ - and  $\gamma$ -Terpineol and terpinenol are represented by the following formulae :



$\beta$ -Terpineol is present in commercial terpineol, and its constitution is determined, like that of  $\alpha$ -terpineol, by oxidation to *p*-methyl tetrahydroacetophenone and then to *p*-tetrahydrotoluic acid. The  $\gamma$ -compound was obtained by Baeyer from dipentene dihydrobromide as described under terpinolene, whilst terpinenol is found in Ceylon cardamom oil and marjoram oil. It has been prepared together with 1.4 terpin by shaking sabinene (p. 227) and thujene (p. 225) with dilute sulphuric acid.



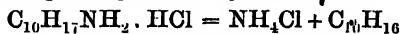
**Carvestrene and Sylvestrene.** The only menthadienes of the meta series are carvestrene, the inactive, and sylvestrene, the active form.

Sylvestrene was first discovered in 1877 by Atterberg in Swedish turpentine (from *Pinus sylvestris*), which is still the principal source of the compound, although it has since been found in dwarf pine oil (from *Pinus montana*) and pine tar oil. It is purified by separating it from the crystalline dihydrochloride by boiling with aniline, and is a curiously stable substance, retaining its optical activity (it is dextro-rotatory) at a temperature of  $250^\circ$ , and undergoing little change by the action of alcoholic sulphuric acid. Both carvestrene and sylvestrene give a characteristic deep blue colour on the addition of a drop of strong sulphuric acid to the terpene dissolved in acetic anhydride, a property which is not shared by any other terpene.

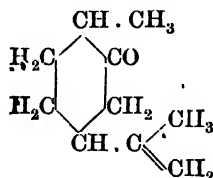
The conversion of sylvestrene into *m*-cymene (see p. 185) and its evident relation to carvestrene point to a common structure for both compounds. This structure will now be considered in the case of carvestrene



Carvestrene is an artificial compound, which was obtained by Baeyer<sup>1</sup> by distilling vestrylamine hydrochloride.

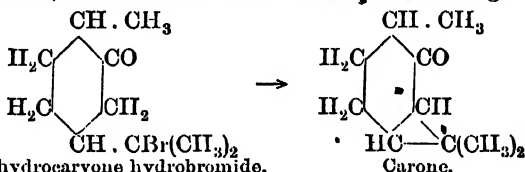


The formation of this base involves a series of complex changes which take us back to dihydrocarvone (p. 190), which, it may be remembered, is the first reduction product of carvone.



Dihydrocarvone.

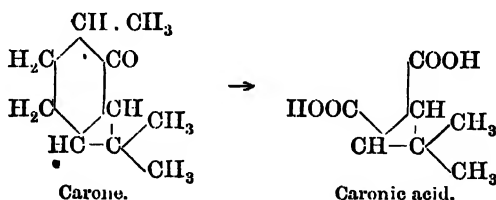
This substance readily forms a hydrobromide from which methyl alcoholic potash removes a molecule of hydrogen bromide; but with the production of an entirely new compound, namely, a saturated bicyclic ketone, to which the name of *carone* has been given.



Dihydrocarvone hydrobromide.

Carone.

Carone has a smell of camphor and peppermint, and is optically active in the sense corresponding to the two carvones from which it may be prepared. The structure of carone is not a mere surmise, but depends on the formation of caronic acid by oxidation; for the same acid has been obtained synthetically by Perkin, jun., and Thorpe,<sup>2</sup> who have identified it as dimethylcyclopropane dicarboxylic acid.



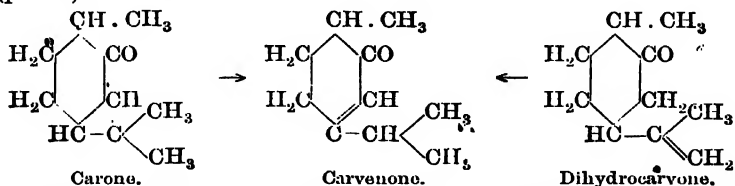
Carone.

Caronic acid.

The existence of such an acid clearly points to a trimethylene ring in carone. An additional proof of its structure is its conversion on heating into carvenone. Carvenone is an unsaturated ketone, which is also obtained by the isomeric change of dihydrocarvone on

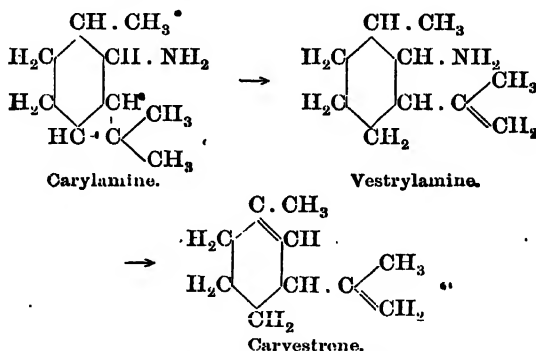
<sup>1</sup> Ber., 1894, 27, 1915, 3485; 1896, 29, 2796.    <sup>2</sup> Trans. Chem. Soc., 1899, 75, 48

boiling with acids (p. 204). The disposition of the double-bond in carvenone has been determined by its conversion into terpinene in the same way that carvotanacetone is converted into  $\alpha$ -phellandrene (p. 201).<sup>1</sup>

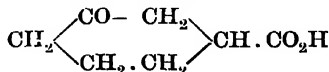


Having settled the structure of carone, the subsequent changes are readily explained.

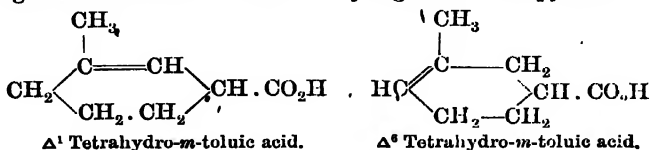
Carone forms an oxime which is reduced to carylamine. If the alcoholic solution of carylamine is saturated with hydrogen chloride gas, the unsaturated base, *vestrylamine*, is formed, from the hydrochloride of which carvestrene is generated



Carvestrene has been synthesized by Perkin, jun., and Tattersall<sup>2</sup> as follows: *m*-hydroxybenzoic acid is first reduced to the hexahydro derivative and subsequently oxidised to cyclohexanone-3-carboxylic acid.



Methyl and hydroxyl are then introduced by Grignard's method in place of the ketonic oxygen, hydroxyl is replaced by bromine, and hydrogen bromide is then removed by digestion with pyridine.



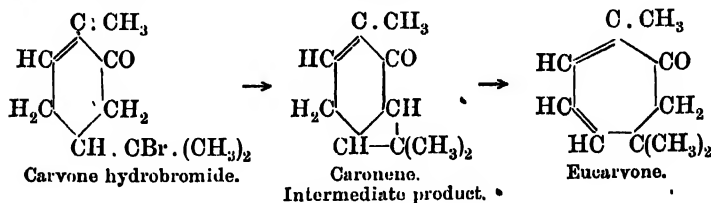
<sup>1</sup> Harries and Majima, *Ber.*, 1908, 41, 2516.

<sup>2</sup> *Trans. Chem. Soc.*, 1907, 91, 480.

Both  $\Delta^1$  and  $\Delta^2$  tetrahydro-*m*-toluic acids are formed, but mainly the former, which is converted into the ester, and the subsequent stages are the same as those described under the synthesis of *p*-menthadiene from tetrahydro-*p*-toluic ester on p. 197.

Later, the tetrahydro-*m*-toluic acid was resolved and the dextro-rotatory terpene thus obtained was identical in every respect with *d*-sylvestrene.<sup>1</sup>

The action of methyl alcohol potash on carvone hydrobromide gives rise to the unsaturated ketone, *eucarvone*,  $C_{10}H_{14}O$ .<sup>2</sup> The fact is referred to as it illustrates in a graphic manner the remarkable reactivity of these cyclic structures, which reaches perhaps its culminating point in the case of camphor. *Eucarvone* is probably a seven-ring complex, in the formation of which caronene probably plays the part of an intermediate product.



The proof of the existence of a seven-ring system is based upon the oxidation of tetrahydroeucarvone to  $\beta$ -dimethyladipic acid.

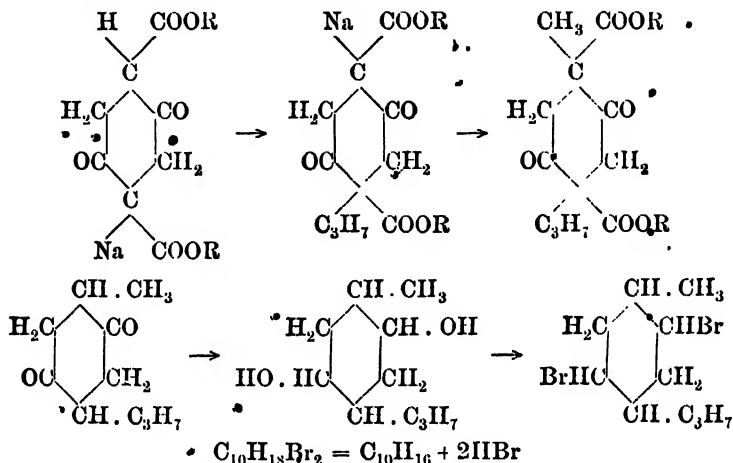
**Synthetic Mono-cyclic Terpenes. Baeyer's Method.** We will conclude our account of the monocyclic terpenes by a short reference to the various attempts which have been made to obtain these compounds synthetically. It is true that several artificial terpenes have been described, but some of them can scarcely be considered as products of constructive synthesis, seeing that the materials have been drawn from natural sources. The first complete and successful synthesis was accomplished by Baeyer<sup>3</sup> as follows: succino-succinic ester was treated with one equivalent of sodium ethoxide and one equivalent of isopropyl iodide, whereby a mono-isopropyl derivative was obtained. The process was repeated, using, however, one equivalent of methyl iodide. This yields a methyl isopropyl derivative, which is then heated with sulphuric acid. Hydrolysis occurs, and at the same time carbon dioxide is evolved with the formation

<sup>1</sup> *Proc. Chem. Soc.*, 1910, 26, 67.

<sup>2</sup> Baeyer, *Ber.*, 1894, 27, 811; 1896, 29, 3; Wallach and Köhler, *Annalen*, 1905, 330, 94; Clarke and Lapworth, *Trans. Chem. Soc.*, 1910, 97, 12.

<sup>3</sup> *Ber.*, 1898, 26, 232.

of methyl isopropylidiketocyclohexane. The ketone was reduced to the alcohol, the hydroxyls replaced by bromine by treatment with strong hydrobromic acid, and, finally, the product heated with quinoline, which removes two molecules of hydrogen bromide. In this way a *p*-menthadiene was produced, which, though closely resembling, was not identical with terpinene. The various changes described above may be represented as follows:



The structure of the terpene in question will obviously depend upon the manner in which the two molecules of hydrogen bromide are detached.

The successful syntheses of dipentene and carvestrene by Perkin, jun., have already been referred to. The same author and his collaborators,<sup>1</sup> by a slight variation of the method, have succeeded in preparing artificially ortho-, meta-, and para-terpenes (menthadienes), terpeneols (menthenols), and the allied menthanols and menthanes (of which the following illustrations must suffice), as well as corresponding derivatives of the cyclopentane series.<sup>2</sup>

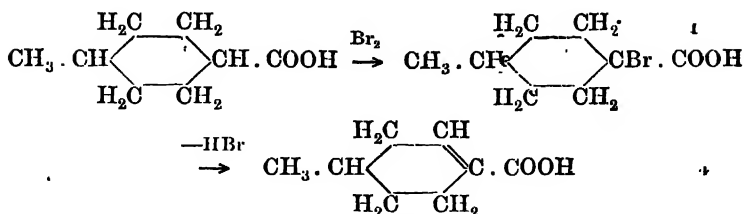
*Perkin's Methods.*<sup>3</sup> There are five general methods which have been employed for this purpose.

1. From the toluic acid by reduction to the hexahydro-derivative and bromination. Removal of hydrogen bromide yields the tetrahydro toluic acid with the double bond in the  $\alpha\beta$  position.

<sup>1</sup> *Trans. Chem. Soc.*, 1905, 87, 639, 661, 1066, 1083.

<sup>2</sup> *Trans. Chem. Soc.*, 1908, 93, 573.

<sup>3</sup> 'Synthesis of the Terpenes,' by W. H. Perkin, *The Perfumery and Essential Oil Record*, 1912, 3, 149.

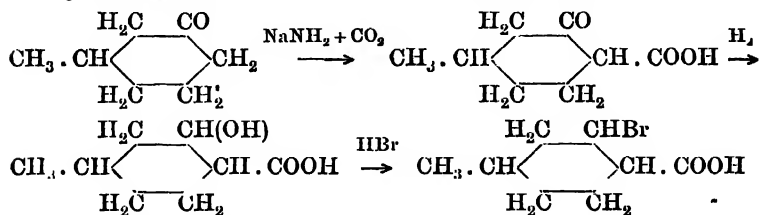


The latter is converted into the ester and the Grignard reagent applied as already described. The resulting menthadiene contains a *conjugated double link*. It is at the stage of unsaturated acid that resolution into the active enantiomorphs has usually been effected through the brucine or strychnine salts. By a process similar to the above, but using the saturated hexahydrotoluic acids, the corresponding menthanols and menthenes have been obtained. Finally, from the menthene the menthane has been prepared by reduction of the menthene hydrogen bromide  $\text{C}_{10}\text{H}_{18}\text{HBr}$  with zinc dust and acetic acid.

2. From the hydroxytoluic acid, which is reduced to the hexahydro derivative and then acted upon with hydrobromic acid, which yields the bromohexahydro acid. The latter is then treated as above. Dipentene was obtained in this way (p. 197).

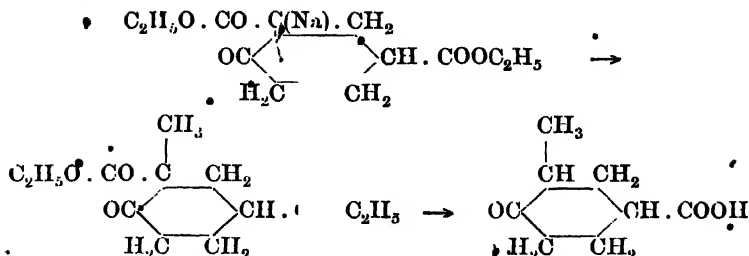
3. From the hydroxybenzoic acid, which is reduced to the hexahydroxy-derivative and then oxidised to the ketonic acid. The ketone group then reacts with the Grignard reagent, and the methyl hydroxy-acid thus formed is converted into the bromo acid, from which hydrogen bromide is then removed. This method has been applied to the synthesis of carvestrene (p. 205).

4. From the methyl cyclohexanone by the combined action of sodamide and carbon dioxide. The ketonic acid is then reduced to the hydroxy acid, which is in turn converted into the bromo-acid.<sup>1</sup>



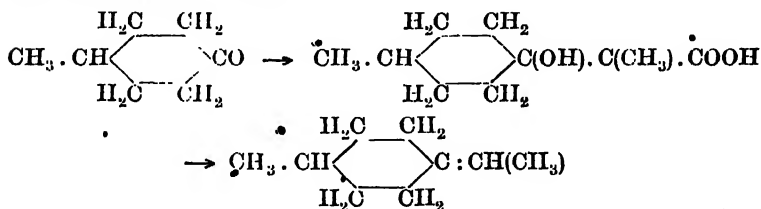
5. From the sodium compound of cyclohexanone 2:4 dicarboxylic ester, which reacts with methyl iodide and gives the methyl derivative; on hydrolysis it is converted into the methyl cyclohexanone carboxylic acid.

<sup>1</sup> *Trans. Chem. Soc.*, 1910, 97, 1756; 1911, 99, 526.

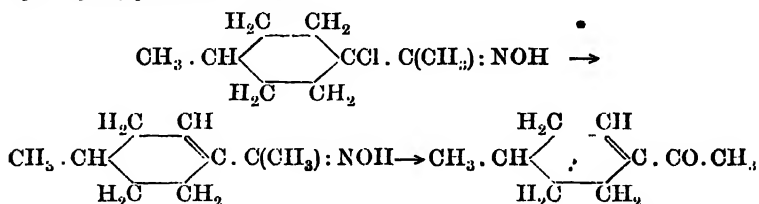


The ketone group is then reduced to the secondary carbinol group and converted into the bromo-derivative in the ordinary way. Carvestrene has been obtained by this method.<sup>1</sup>

*Perkin-Wallach Method.*<sup>2</sup> The cycloketone is combined with  $\alpha$ -bromopropionic ester in presence of zinc (Reformatsky's reaction, Part I, p. 217). The free acid is decomposed on heating, thereby losing water and carbon dioxide.



The latter is converted into the nitrosochloride, from which, by elimination of hydrogen chloride, the oxime is formed, and this, on hydrolysis, yields the ketone.

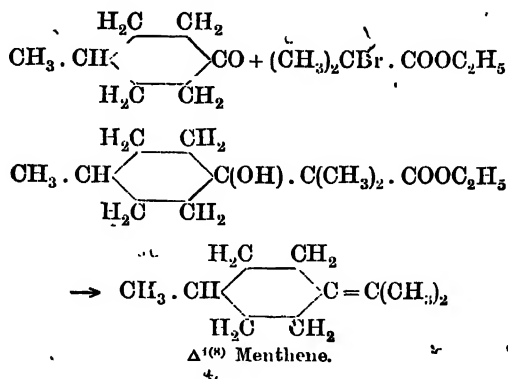


By means of the Grignard reagent the latter is converted into the menthenol and the menthadiene. Menthadienes prepared in this way contain a conjugated double bond.

Using a similar reaction to the above—that is, by the action of  $\alpha$ -bromisobutyric ester on the cyclic ketone—a  $\Delta^{4(10)}$  menthene has been obtained by Wallach.

<sup>1</sup> Perkin and Fisher, *Trans. Chem. Soc.*, 1908, 93, 1876.

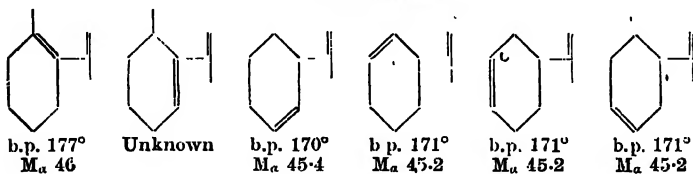
<sup>2</sup> *Trans. Chem. Soc.*, 1910, 97, 1429; 1911, 99, 118. *Annalen*, 1910, 374, 198; 1911, 379, 131.



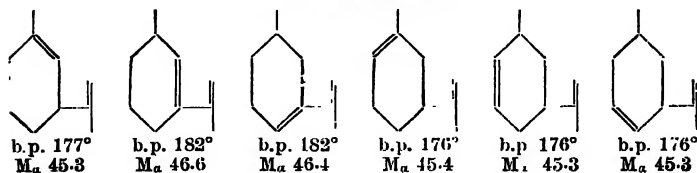
It should be pointed out that the above method has been utilized by Wallach for introducing an unsaturated group in place of the methyl side-chain, as described on p. 200.

The following is a list of *o*-, *m*-, and *p*-menthadienes obtained by one or other of these methods, with their boiling-points and molecular refractivities:

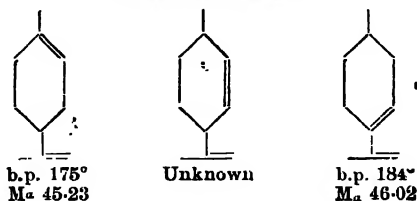
*Ortho-Menthadienes.*



*Meta-Menthadienes.*



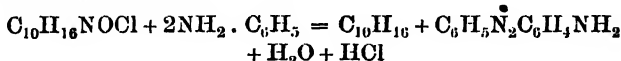
*Para-Menthadienes.*



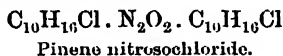
It will be observed in the above table that the menhadienes with conjugated double bonds exhibit a higher boiling-point and a refractivity above the calculated value for two double bonds ( $M_a = 45.24$ ). This increase of refractivity or exaltation may serve as a convenient method in doubtful cases of fixing the position of the double bonds (see Part II, p. 28). Conjugation also manifests itself in chemical behaviour, for those compounds in which it occurs unite with only one molecule instead of two molecules of bromine, hydrogen chloride, and bromide (see Part I, p. 133).

### THE BI-CYCLIC TERPENES

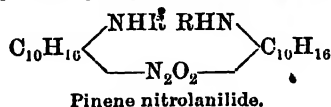
**Pinene.**  $\alpha$ -Pinene is the most widely distributed of all the terpenes. It is a common constituent of most essential oils, and is specially abundant in the resinous exudations of different species of pinus, from which it is obtained by distillation in the form of turpentine oil. Two optically active modifications are known, the *d*-compound, sometimes distinguished by the name *australene*, being found in American turpentine (from *Pinus palustris* or *australis*), and also in German, Russian, and Swedish turpentine (from *Pinus sylvestris*) and in many essential oils, and the *l*-compound or *terebenthene* in French turpentine (from *Pinus pinaster*), English pine needle oil, hemlock oil, &c. It is still doubtful whether the two are strictly enantiomorphous. Pinene from pine is obtained by first fractionating turpentine oil and then converting it into the crystalline nitrosochloride, from which, by boiling with aniline in alcoholic solution, the pure but inactive compound is regenerated. In this reaction aminoazobenzene is also formed.



Pinene contains one double bond; for, as just stated, it forms a compound with one molecule of nitrosyl chloride, to which Baeyer assigns the double molecular formula:



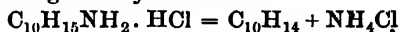
This compound forms a series of *nitrolamides* by the exchange of chlorine for primary basic groups having the general formula:



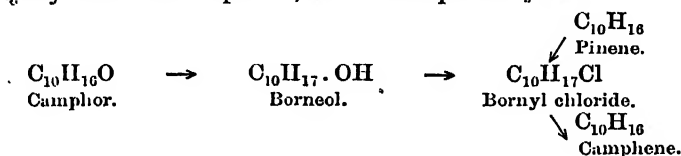
Sodium alcoholate removes hydrogen chloride from the nitrosochloride, forming *nitrosophene*,  $\text{C}_{10}\text{H}_{16}\text{NO}$ , which can be reduced to



*pinylamine*,  $C_{10}H_{15}.NH_2$ . The latter is readily converted into *p*-cymene by distilling the hydrochloride.



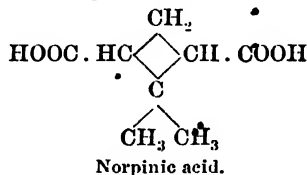
In addition to the nitrosochloride, pinene forms crystalline additive compounds with one molecule of dry hydrogen chloride and bromide,  $C_{10}H_{16}.HCl$  and  $C_{10}H_{16}.HBr$ , from which the original product cannot be regenerated; for on removing the halide acid with quinoline or sodium acetate a new terpene, namely *camphene*, is produced. Now *camphene* is closely related to *camphor*, for *camphor*  $C_{10}H_{16}O$  on reduction forms *borneol*  $C_{10}H_{17}OH$ , which is a secondary alcohol giving with phosphorus chloride *bornyl chloride*, and *bornyl chloride* is identical with the hydrochloride obtained by the action of dry hydrogen chloride on pinene. It is, however, not the true hydrochloride of pinene, as we shall presently see.



When it is remembered that pinene is converted by moist hydrogen chloride into dipentene dihydrochloride, and by alcoholic sulphuric acid and other reagents into such varied products as terpin hydrate, terpineol, cineol, dipentene, terpinolene and terpinene, it is easy to realize that the structure of pinene is a pivot upon which that of nearly the whole terpene and camphor family turns. A correct interpretation of its structure is therefore of the highest importance, but its remarkable reactivity rather enhances than diminishes the difficulty of the problem. The successful solution of the problem is mainly due to the combined labours of Baeyer, Tiemann, and Wagner. As in other cases the most valuable information has been gathered from the behaviour of pinene on oxidation. Let us follow the steps in this process of dismemberment. By the action of free oxygen and water on pinene, Sobrero obtained the crystalline *pinol hydrate* or *sobrerol*,  $C_{10}H_{16}(OH)_2$ , which on warming with dilute hydrochloric acid is converted into *pinol*,  $C_{10}H_{16}O$ . Both *sobrerol* and *pinol* are neutral compounds whose constitution will be explained presently. By means of permanganate solution, Baeyer<sup>1</sup> obtained a much more complete series of oxidation products. He found among the first products two acids, namely *α-pinonic acid*,  $C_{10}H_{16}O_3$ , and *pinoyl formic acid*,  $C_{10}H_{14}O_3$ . The first is a ketonic monobasic acid, and the second a ketonic dibasic acid. As both acids yield on further oxidation the same dibasic *pinic acid*,

<sup>1</sup> Ber., 1896, 29, 1907; see also Henderson and Agnew, Trans. Chem. Soc., 1909, 95, 289.

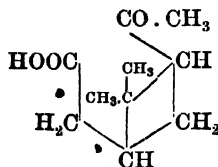
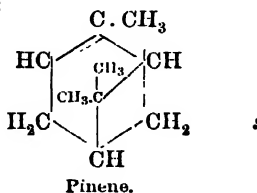
$C_7H_{12}(COOH)_2$ , Baeyer<sup>1</sup> concluded that they contain respectively a methyl ketone— $CO \cdot CH_3$ , and an  $\alpha$ -ketonic acid— $CO \cdot COOH$  group. Pinic acid is very stable; but by first converting it into hydroxypinic acid (by bromination and subsequent hydrolysis), it may be further oxidised to the lower homologue *norpinic acid*,  $C_6H_{10}(COOH)_2$ , a dibasic acid which resists further oxidation. The structure of norpinic acid, which is the key to the problem, is regarded by Baeyer as a cyclobutane derivative.



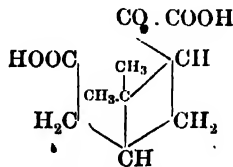
Like carone which gives caronic acid, and therefore contains a cyclopropane nucleus, so pinene must contain a bridged ring, of which one part consists of four carbon atoms.

Both  $\alpha$ -pinonic and pinoyl formic acids are very unstable in acid solution, and like pinene itself are readily transformed by hot dilute sulphuric acid into isomeric compounds, a change which Baeyer ascribes to the rupture of the cyclobutane ring. Pinonic acid gives the methyl ketone of homoterpenylic acid, which Wallach had previously obtained by the oxidation of terpineol (p. 193), whereas pinoyl formic acid is converted into homoterpenyl formic acid, which in turn can be oxidised to homoterpenylic and terpenylic acid of known constitution.

Adopting Wagner's formula for pinene, Baeyer explains the various steps as follows :

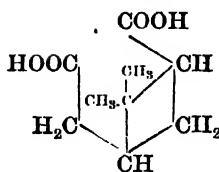


$\alpha$  Pinonic acid.

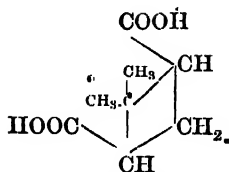


Pinoyl formic acid.

<sup>1</sup> Ber., 1896, 29, 1907.

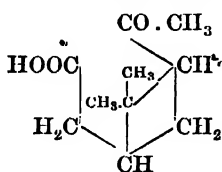
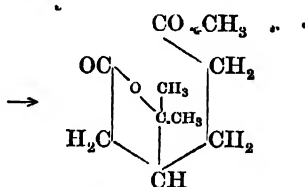
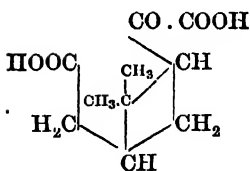


Pinic acid.

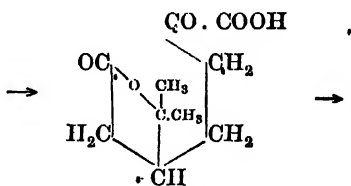


Norpinic acid.

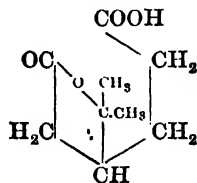
$\alpha$ -Pinonic acid is therefore produced by the rupture of the double bond in pinene. The decomposition of  $\alpha$ -pinonic and pinoyl formic acid by sulphuric acid is represented as follows:

 $\alpha$  Pinonic acid.Methyl ketone of  
Homoterpenylic lactone.

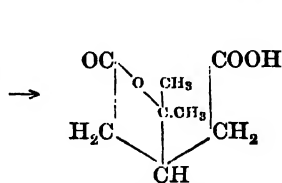
Pinoyl formic acid.



Homoterpenyl formic acid.



Homoterpenylic acid.



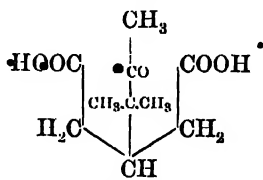
Terpenylic acid.

This view of the structure of pinene, which fits in so neatly with the facts described above, is opposed to the observations of Tiemann and Semmler.<sup>1</sup> Tiemann and Semmler, like Baeyer, submitted pinene to the oxidising action of permanganate, and obtained a saturated ketonic acid, pinonic acid, which, on further oxidation

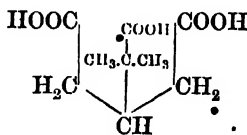
<sup>1</sup> Ber., 1896, 29, 529, 3027.

with chromic acid mixture, gave a dibasic ketonic acid, isoketocamphoric acid,  $C_{10}H_{10}O_5$ , isocamphoronic acid,  $C_9H_{14}O_6$ , and terebic acid,  $C_7H_{10}O_4$ , whilst alkaline permanganate converted it into dimethyltricarballic acid.

Now, the constitution of isoketocamphoric acid is known, partly from its chemical properties but mainly from its relation to isocamphoronic acid, which it yields on oxidation,<sup>1</sup> and whose structure has been established beyond question by its synthesis by Perkin<sup>2</sup> (Part I, p. 203). The two compounds must be represented as follows:

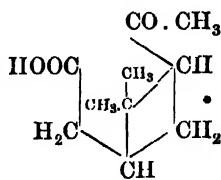
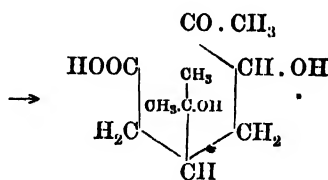


Isoketocamphoric acid.

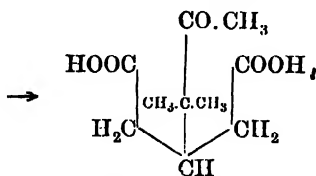


Isocamphoronic acid.

It is impossible by any device to rupture the bonds in Wagner's pinene formula so as to yield either of these acids, and the only alternative is to suppose that an internal rearrangement of the molecule of  $\alpha$ -pinonic acid occurs during oxidation after the following fashion:

 $\alpha$ -Pinonic acid.

Intermediate product.



Isoketocamphoric acid.

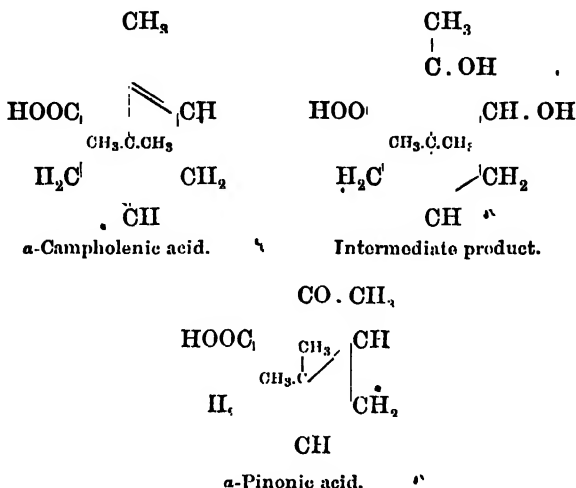
Another difficulty encountered by Wagner's formula is the formation of pinonic from  $\alpha$ -campholenic acid. The latter acid is obtained

<sup>1</sup> *Trans. Chem. Soc.*, 1899, 75, 900.

<sup>2</sup> Perkin, jun., *Proc. Chem. Soc.*, 1900, 16, 214.

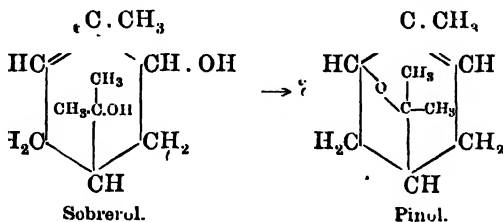
from camphor, and its structure, which is discussed on p. 251. presents no ambiguity.

The explanation which is here put forward, is based upon the pinacone-pinacolone change (Part II, p. 357).

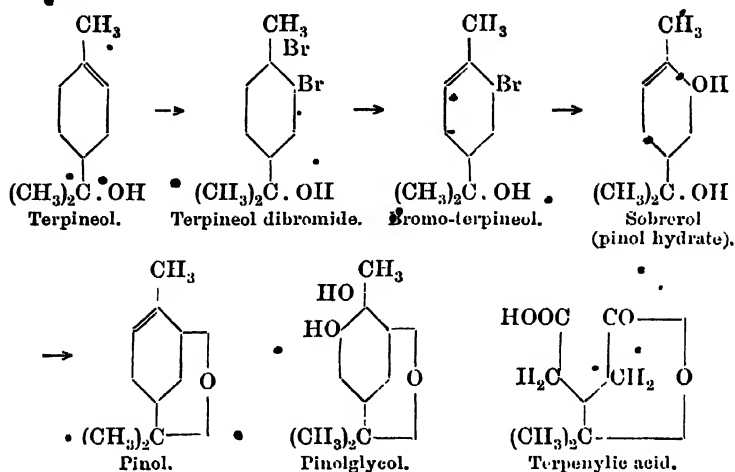


Having now laid the foundation upon which the formula for pinene rests, and attempted to adjust its structure to that of isocamphoronic and  $\alpha$ -campholenic acid, let us examine for a moment its relation to the first products of oxidation, namely, sobrerol and pinol, and finally to the allied terpene, dipentene.

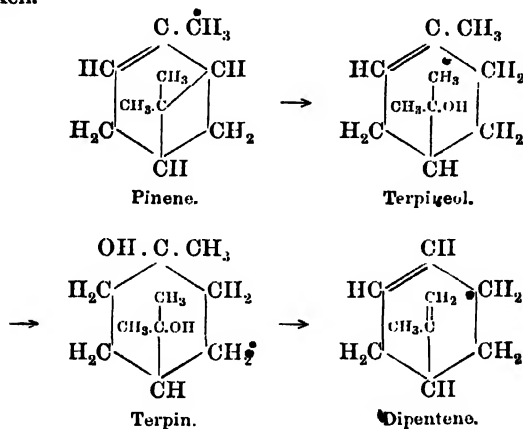
The formation of sobrerol is explained by the rupture of the cyclobutane ring, that of pinol by the loss of water from sobrerol and formation of an inner ether. As both compounds are oxidised by permanganate, one to pinolglycol,  $\text{C}_{10}\text{H}_{16}(\text{OH})_2\text{O}$ , and the other to sobrerithritol,  $\text{C}_{10}\text{H}_{16}(\text{OH})_4$ , they must still possess a double bond. Furthermore, pinolglycol gives terpenylic acid on oxidation. These results are expressed by the following formulae:



Finally, the structure of sobrerol (pinol hydrate) and pinol has been determined by their formation from terpineol (m. p. 35°) in the following way: Terpineol was converted into the dibromide, hydrogen bromide removed, and the remaining bromine atom replaced by hydroxyl.<sup>1</sup>

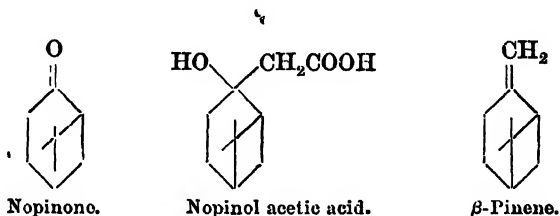


In the formation of dipentene, pinene passes through the intermediate stages of terpineol and terpin, and here again the inner ring is broken.



<sup>1</sup> Wallach, *Annalen*, 1893, 277, 133.

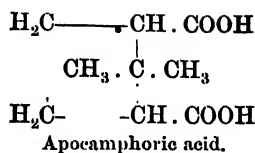
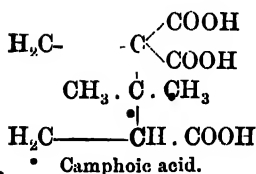
In addition to ordinary or  $\alpha$ -pinene, a small quantity of a second isomer,  $\beta$ -pinene, is present in turpentine. It differs from the  $\alpha$ -compound in always occurring as the laevo form in both active varieties of turpentine. Moreover, on oxidation it gives a cyclic ketone, *nopinone*  $C_9H_{14}O$ , with the loss of an atom of carbon, and replacement of two hydrogen atoms by oxygen, in the same way that  $\beta$ -terpinene and  $\beta$ -phellandrene yield cyclic ketones under similar conditions. That  $\beta$ -pinene differs from the  $\alpha$ -isomer by containing an unsaturated  $=CH_2$  side has been definitely proved by its synthesis from *nopinone* by Wallach's method, just as  $\beta$ -phellandrene is obtained from isopropylcyclohexanone (p. 229).<sup>1</sup>



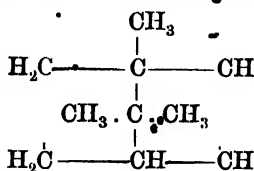
**Camphene and Bornylene.** Camphene is a solid terpene. It is dextro-rotatory in ginger, rosemary, and spike oil, and laevo-rotatory in citronella and valerian oil, and in French and American turpentine. Berthelot obtained the same two active forms from *d*- and *l*-pinene by the action of dry hydrogen chloride, which converts them into the corresponding *d*- and *l*-bornyl chlorides (p. 219). It is also obtained from borneol and the isomeric isoborneol (p. 222) by the action of dehydrating agents. Its mode of preparation would naturally suggest that camphene contained a double bond, and this is supported by the following evidence: the molecular refraction constant observed by Brühl, the formation of a hydrochlorocamphene,  $C_{10}H_{17}Cl$ , with hydrogen chloride gas, of a dibromide  $C_{10}H_{16}Br_2$ , with bromine, and of a glycol,  $C_{10}H_{16}(OH)_2$ , by the action of dilute permanganate. Oxidised with nitric acid it yields camphoic acid, which, on heating, loses carbon dioxide and forms apocamphoric acid, which has been synthesised by Komppa.<sup>2</sup>

<sup>1</sup> Wallach, *Annalen*, 1907, 356, 227.

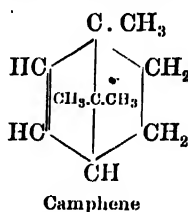
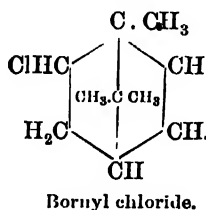
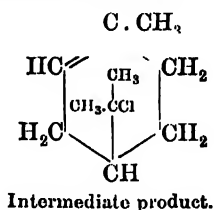
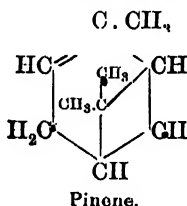
<sup>2</sup> *Ber.*, 1901, 34, 2472.



It would appear from this array of simple facts that the structure of camphene would offer no difficulty, and indeed for a long time the following formula was accepted without question :



It explains primarily its relation to camphor and borneol (see p. 245), and is consistent with the facts enumerated above, including its formation from pinene, which is expressed as follows :

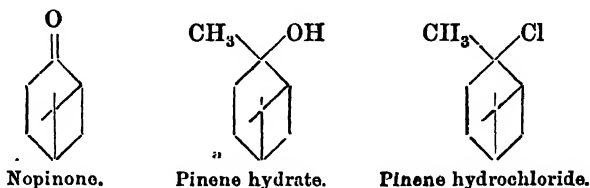


Nevertheless, this view is contested by Wagner and Semmler, whose objection is based on the nature of the halogen compounds from which camphene is prepared.

In the first place, the additive compounds of pinene and camphene with hydrogen chloride are not identical, for the pinene compound is much the more stable. The hydrochloride obtained from pinene resists the action of boiling water to a great extent, whilst the camphene compound almost completely loses hydrogen chloride by this treatment.



Yet neither substance is the true hydrochloride of pinene, which has been prepared by Wallach<sup>1</sup> from nopinone by means of the Grignard reagent, which gives pinene hydrate and treatment of the latter with phosphorus pentachloride.



Corresponding to the first two hydrochlorides are two borneols, known as borneol and isoborneol, both of which are obtained simultaneously by the reduction of camphor with sodium in alcoholic solution. By the action of phosphorus pentachloride on isoborneol, or hydrogen chloride on its alcoholic solution, camphene hydrochloride is produced. Moreover, camphene combines with organic acids in presence of sulphuric acid to form esters of isoborneol. It seems clear, therefore, that camphene and isoborneol are structurally related. Wagner and Brykner<sup>2</sup> have now shown that, when phosphorus pentachloride reacts with borneol, a mixture of bornyl and isobornyl chloride is produced, but mainly the latter, which proves to be a secondary product of the action of hydrogen chloride on the camphene formed by the dehydration of borneol. If in place of phosphorus pentachloride, dry hydrogen iodide is allowed to react with borneol, bornyl iodide is formed, from which alcoholic potash liberates a new terpene, called *bornylene*. Bornylene may also be obtained from borneol by the xanthic ester method described on p. 227, and in other ways.<sup>3</sup> As bornyl iodide is identical with the hydriodide of pinene, there is little doubt that the hydrochloride of pinene is the true halogen ester of borneol.

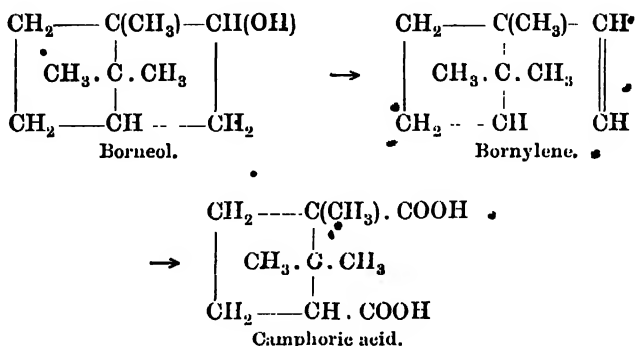
As both borneol and isoborneol give camphor on oxidation, and until recently were thought to yield camphene by dehydration, the differences between them were regarded as of a stereochemical nature. With the discovery of bornylene and the great divergence which is now recognized in the properties not only of bornylene and camphene but of borneol and isoborneol, this view has undergone a change, and

<sup>1</sup> *Annalen*, 1907, 356, 227.

<sup>2</sup> *Ber.*, 1899, 32, 2320; 1900, 33, 2121.

<sup>3</sup> Brodit and Sandkuhl, *Annalen*, 1909, 366, 1; Henderson and Caro, *Trans. Chem. Soc.*, 1912, 101, 1416.

they are looked upon as structurally distinct. Bornylene has received the formula originally attached to camphene, and best indicates its relation to borneol and to camphoric acid, which it readily yields on oxidation.



The structure of camphene is still doubtful. By carefully regulated oxidation, Wagner obtained a *camphene glycol*,  $\text{C}_{10}\text{H}_{16}(\text{OH})_2$ , which is, however, quite distinct from camphor glycol. From camphene glycol a series of oxidation products have been prepared, among which a dibasic acid, *camphenecamphoric acid*,  $\text{C}_{10}\text{H}_{16}\text{O}_4$ , a hydroxy acid, *camphenylic acid*,  $\text{C}_{10}\text{H}_{16}\text{O}_3$ , and a ketone, *camphenilone*,  $\text{C}_9\text{H}_{14}\text{O}$ , have been isolated and studied.

In another direction the action of chromylchloride has produced an aldehyde *camphenilan aldehyde*,  $\text{C}_9\text{H}_{15}\text{CHO}$ , from which camphenilanic acid has been prepared. This acid has been converted successively into a bromo and a hydroxy acid, the latter being identical with camphenilone. In the present state of the camphene problem it seems useless to multiply the names and formulae of its derivatives, and the subject must be left until further research has thrown new light upon it.

The synthesis of camphenilone by Bouveault and Blanc,<sup>1</sup> and by Komppa, by the distillation of the lead salt of camphenic acid,<sup>2</sup> and further, that of camphenic acid by Lipp,<sup>3</sup> point unmistakably to the formula for camphene proposed by Wagner<sup>4</sup> and adopted later by Semmler.<sup>5</sup>

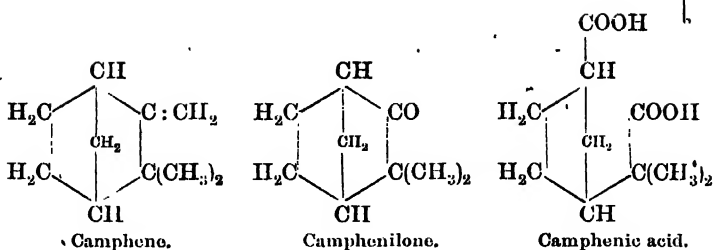
<sup>1</sup> *Compt. rend.*, 1908, 147; 1314.

<sup>2</sup> *Ber.*, 1914, 47, 871.

<sup>3</sup> *Ber.*, 1914, 47, 934 (foot-note).

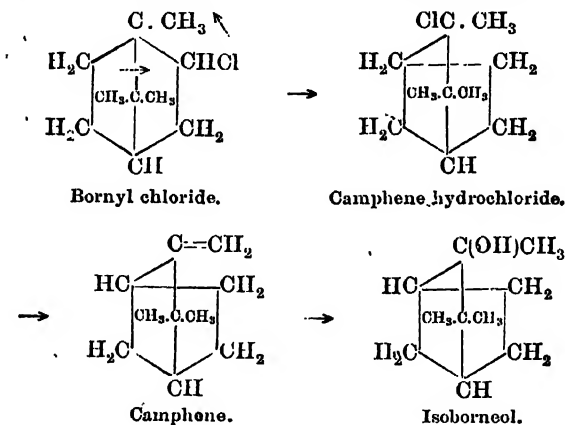
<sup>4</sup> *Ber.*, 1900, 33, 2124.

<sup>5</sup> *Ber.*, 1909, 42, 246, 962.



This structure agrees well with the conversion of camphene into a mono-ozonide (Part I, p.120) and the formation of cyclopropane ring with diazoacetic ester (Part I, p. 204).

Its relation to bornyl chloride may be represented as follows:<sup>1</sup>

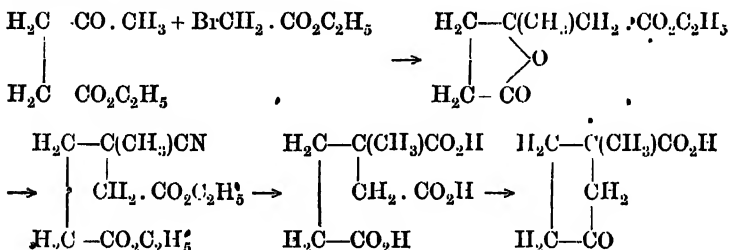


**Fenchene.** Two active fenchenes are prepared from corresponding *fenchones* much in the same way that bornylene or camphene are prepared from camphor. It is therefore necessary to preface a description of their preparation with some account of fenchone. Fenchone, C<sub>10</sub>H<sub>16</sub>O, was discovered by Wallach in 1890. It is dextrogyrate in fennel oil (*foeniculum vulgare*) and laevogyrate in thuja oil, in which it occurs with *thujone*, an isomeric ketone. Fenchone has similar properties to camphor (p. 233), but is a liquid. It is a ketone, and forms an oxime, but contains no CH<sub>2</sub>.SO group, since it yields no hydroxymethylene compound. On reduction it gives a secondary alcohol, *fenchyl alcohol*, C<sub>10</sub>H<sub>18</sub>O, from which

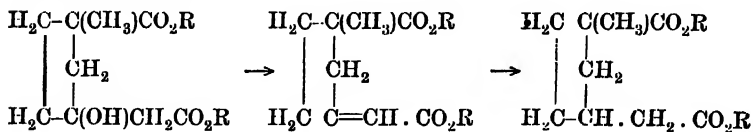
<sup>1</sup> Harries and Palmén, *Ber.*, 1910, 43, 1432.

various terpenes of the formula  $C_{10}H_{16}$ , the fenchenes, can be obtained by the aid of dehydrating agents. Oxidising agents convert fenchene into apocamphoric acid, whilst fenchone gives isocamphoric acid (p. 245). Fenchoneoxime, like camphoroxime, loses water on heating with acids, and gives unsaturated fencholenic nitriles which, on hydrolysis, are converted into  $\alpha$ - and  $\beta$ -fencholenic acids. These facts have found expression in a formula for fenchone which has been confirmed by its synthesis by Ruzicka, as follows:<sup>1</sup>

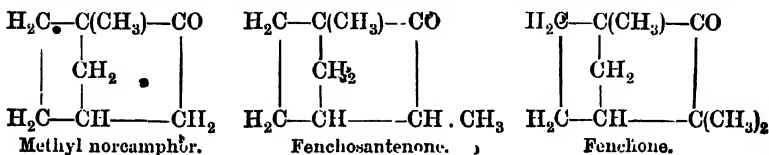
Levulinic ester and bromacetic ester are condensed by Reformatsky's reaction, and the lactonic ester thus obtained is converted into the nitrile by means of potassium cyanide. On hydrolysis the resulting, tricarboxylic ester is heated with sodium and benzene, whereby a pentamethylene derivative is obtained. The following represent the above changes:



The ester of this acid is condensed with bromacetic ester, and by eliminating water from the product an unsaturated ester was obtained, which was then reduced."

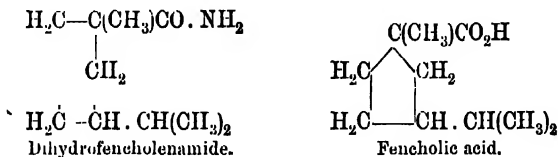


By distilling the lead salt of the last acid, methyl norcamphor is formed. On methylating the latter with methyl iodide and sodamide, a mixture of fenchone and fenchosantenone was obtained.



<sup>1</sup> Ber., 1917, 50, 1362.

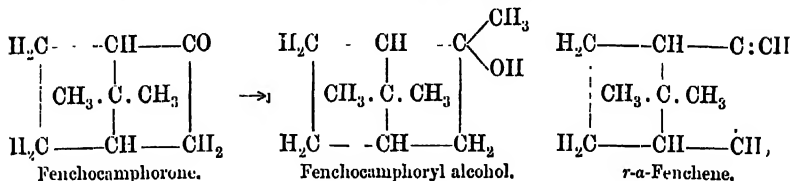
This formula agrees with the structure of *dihydrofencholenamide*, which Bouveault and Levallois <sup>1</sup> obtained by synthesis,



and also from the behaviour of fenchone on heating with caustic potash, when it passes smoothly into *fencholic acid* (methyl isopropyl cyclopentane carboxylic acid).<sup>2</sup>

In addition to the action of dehydrating agents on the fenchyl alcohols, the fenchenes may be prepared by removing hydrogen chloride from the fenchyl chlorides, which are in turn formed from the alcohols by the action of phosphorus chloride. In this way at least four fenchenes are obtained, two from each of the active fenchyl alcohols, which are distinguished by the symbols *d*, *l*- $\alpha$ -, and *d*, *l*- $\beta$ -, according to the method of preparation, of which *l*- $\alpha$ -fenchene from *l*-fenchone is best known. In its preparation *d*-fenchone is reduced to fenchyl alcohol, which is laevo-rotatory. If carefully cooled when phosphorus chloride is added, a strongly laevo-rotatory fenchyl chloride is formed, from which *l*- $\alpha$ -fenchene is obtained by removing hydrogen chloride with aniline. It is a curious fact that if the fenchyl alcohol is not cooled during the action of the phosphorus compound, the product is dextro-rotatory.

The synthesis of *r*- $\alpha$ -fenchene has recently been effected by Komppa and Roschier<sup>3</sup> from fenchocamphorone, which had been previously synthesized (see p. 225), by the action of magnesium methyl iodide and distillation of the resulting alcohol.



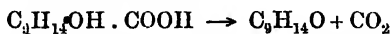
This view of the structure explains the various oxidation products obtained by Wallach. Fenchene yields a hydroxyfenchenic acid,  $\text{C}_{10}\text{H}_{16}\text{O}_3$ , which, like  $\alpha$ -hydroxy acids, is converted by means of

<sup>1</sup> *Compt. rend.*, 1908, 146, 180.

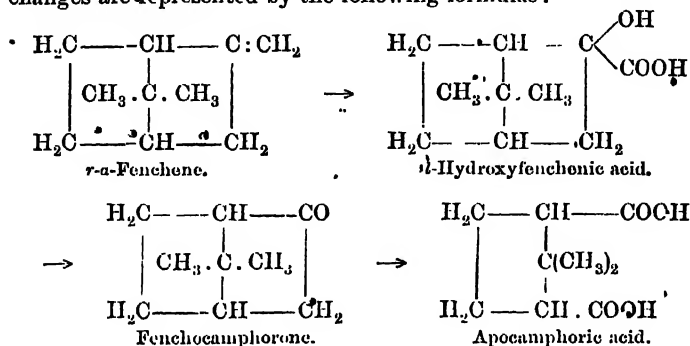
<sup>2</sup> *Annalen*, 1909, 369, 71.

<sup>3</sup> *Abstr.*, 1916, i, 466.

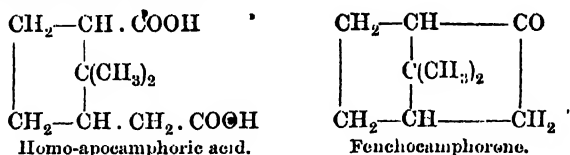
lead peroxide and sulphuric acid into a ketone, fenchocamphorone, with elimination of carbon dioxide.



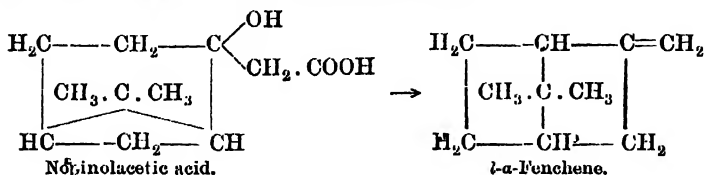
Fenchocamphorone yields, on the one hand, an oxime which readily loses water and passes into fenchocamphonitrile,  $C_9H_{13}CN$ , and, on the other, passes by oxidation into apocamphoric acid. These changes are represented by the following formulae:



Komppa<sup>1</sup> has synthesized fenchocamphorone by heating the lead salt of homo-apocamphoric acid.



The presence of an exocyclic double bond in *l*- $\alpha$ -fenchene is proved by its formation, together with  $\beta$ -pinene, from nopinolacetic acid (p. 218) when acted on by dehydrating agents.<sup>2</sup>



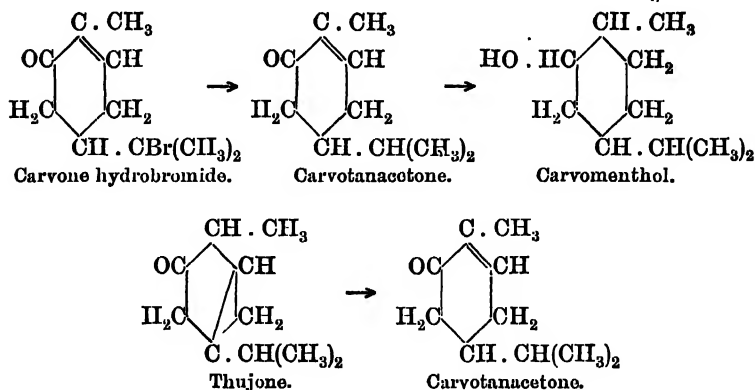
**Thujene.** Thujene, like fenchene, is obtained from a corresponding ketone, *thujone* or *tanacetone*. According to Wallach, thujone exists in isomeric forms. Together with *l*-fenchone it occurs

<sup>1</sup> Ber., 1914, 47, 938

<sup>2</sup> Wallach, 1907, *Annalen*, 357, 53; 2908, 363, 3.

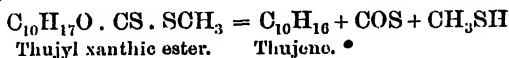
as the chief constituent of thuja oil, but is also found in wormwood and sage oil, and is laevo-rotatory. The  $\beta$ -compound occurs mainly in tansy and wormwood oil, and is dextro-rotatory. Chemically they are very similar.

Thujone is separated as the bisulphite compound. As its constitution is bound up with that of thujene, we will consider the properties of the former first. Thujone forms a semicarbazone and an oxime, which gives *thujylamine*,  $C_{10}H_{17}NH_2$ , on reduction. On distilling the hydrochloride of the base, thujene is formed. Thujone is saturated, i.e. it yields no additive products, and therefore presumably is a bi-cyclic ketone of the nature of carone, a view which is confirmed by its high molecular refractivity (44.78), which is above the normal (44.11) (see Part II, p. 27). Its resemblance to carone is further demonstrated by the action of heat, which converts it into the isomeric carvotanacetone in the same manner that carone yields carvenone (p. 205). Now the structure of carvotanacetone is known and is derived partly from its formation from carvone hydrobromide by reduction and partly by its conversion into carvomenthol, the isomer of menthol. These facts are expressed by the following structural formulae:

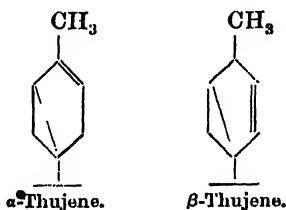


Wallach found that thujone undergoes a further isomeric change on boiling with sulphuric acid, yielding an unsaturated ketone, *isothujone*, which, according to Semmler, contains a cyclopentane nucleus; but the consideration of this compound need not detain us. Thujene combines with hydrogen chloride and gives terpinene dihydrochloride, and is transformed into terpinenol on shaking with dilute sulphuric acid (p. 203). Thujene can also be prepared from

thujone by means of the xanthic ester method elaborated by Tschugaëff,<sup>1</sup> and which seems capable of a very wide application. Thujyl alcohol, which is obtained from thujone by reduction, is converted into the xanthic methyl ester by treating the sodium compound of the alcohol with methyl iodide and carbon bi-sulphide. It is then decomposed by dry distillation and gives the terpene :



According to Tschugaëff, the thujene obtained in this way ( $\beta$ ) is isomeric and not identical with the first, and considers that they stand in the following relationship :



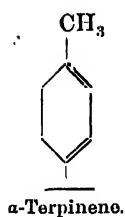
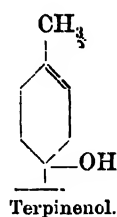
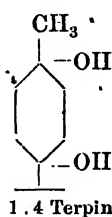
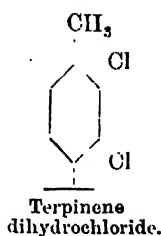
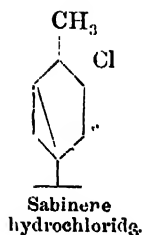
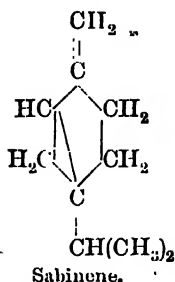
**Sabinene** occurs in savin oil, and also in Ceylon cardamom oil and marjoram oil. It shows a molecular refractivity ( $M = 44.88$ ) somewhat above the normal ( $M = 43.53$ ), and therefore probably contains a cyclopropano ring. Our knowledge of its properties and structure is mainly due to the researches of Wallach.<sup>2</sup> It presents not only an example of those curious isomeric changes to which these substances are peculiarly disposed and by which they are transformed into other terpenes; but sabinene has furnished material for the synthesis of several terpenes whose structure has been ascertained by its aid.

With dry hydrogen chloride sabinene forms a monohydrochloride, but with the moist acid it gives terpinene dihydrochloride. When shaken with cold, dilute sulphuric acid it yields terpinenol and 1.4 terpin (p. 228), and when heated with dilute sulphuric it is converted into terpinene. The last may also be obtained directly from sabinene and its hydrate. These transformations are readily explained as follows :

<sup>1</sup> *J. Russ. Phys. Chem. Soc.*, 1904, 36, 988.

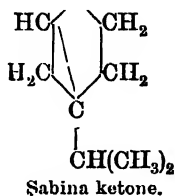
<sup>2</sup> *Annalen*, 1907, 350, 165





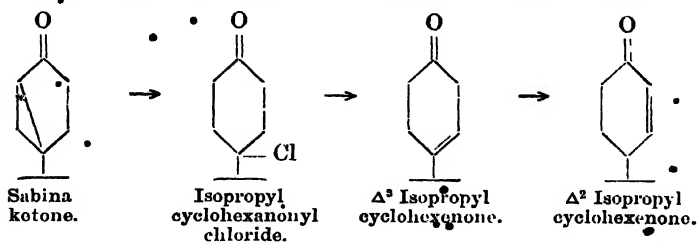
When reduced with hydrogen in presence of platinum black it gives the same hydrocarbon as thujene, which has been termed *thujane*.<sup>1</sup> When sabinene is oxidised with permanganate it loses an atom of carbon and yields a ketone,  $C_9H_{14}O$ , known as sabina ketone. From this it follows that the carbon of the unsaturated side-chain is removed and replaced by oxygen.

O



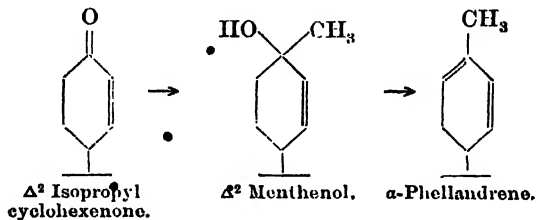
When sabina ketone is acted upon with hydrochloric acid it yields a hydrochloride from which hydrogen chloride may be again removed by warming with aniline. The original ketone is not,

however, regenerated, but two new cyclic ketones, which have been identified as  $\Delta^2$  and  $\Delta^3$  isopropyl cyclohexenones, are produced.



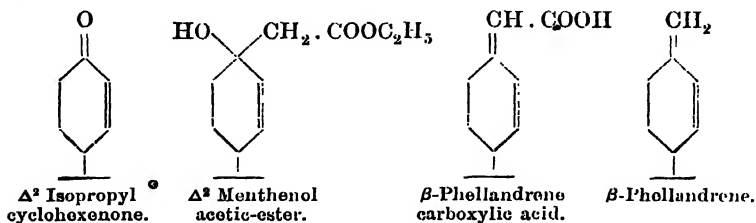
The two latter have been utilized for the synthesis of  $\alpha$ -terpinene and  $\alpha$ - and  $\beta$ -phellandrene.

$\alpha$ -Phellandrene was obtained from the  $\Delta^2$  cyclohexenone by means of the Grignard reagent and subsequent removal of water.

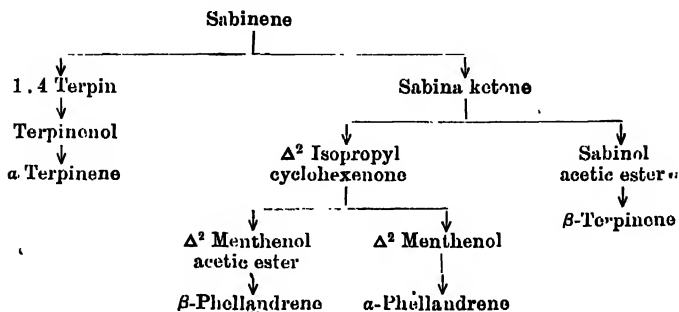


The formation of  $\beta$ -terpinene has already been described (p. 200); that of  $\beta$ -phellandrene is of a similar character.

$\Delta^2$  Isopropyl cyclohexanone is combined with  $\alpha$ -bromacetic ester in presence of zinc. The resulting hydroxy-ester is hydrolysed and heated with acetic anhydride, whereby water is eliminated. The unsaturated acid which is formed is heated, when carbon dioxide is split off and  $\beta$ -phellandrene is formed.

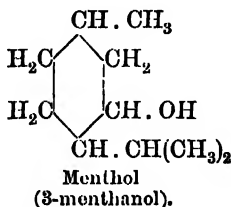
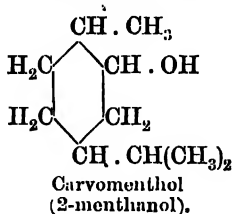


The relationships of sabinene to the other terpinenes may be tabulated in the following way:



We have not exhausted the subject of the terpenes, nor even enumerated all the known members. We have been content to give a sufficiently broad *résumé* of this branch of organic chemistry to enable the reader to realize the nature and remarkable reactivity of these substances.

**The Menthanol.** The menthanol include the hydroxy-derivatives of hexahydrocymene. The best-known member of the group, *l*-menthol (or 3-menthanol), is the principal constituent of peppermint oil, and has also been prepared from pulegone (see p. 231). On oxidation it yields the ketone, menthone, and is therefore a secondary alcohol. A *d*-menthol has been obtained by Kondakon and Tschugaeff<sup>1</sup> by reducing the menthone from bucco oil. As menthone on further oxidation yields  $\beta$ -methyl adipic acid, the position of the ketone group is the same as that in pulegone. The isomeric carvomenthol is obtained from carvone, and the position of the hydroxyl group is thereby determined.



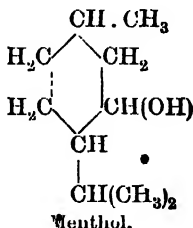
Certain synthetic menthapyols have also been prepared by Perkin from the hexahydrotoluic acids by means of the Grignard reaction, as already described (p. 197). They therefore contain a tertiary hydroxyl in the isopropyl side-chain.

<sup>1</sup> J. Russ. phys., chem. Ges., 1910, 42, 714.

## THE CAMPHORS

It has already been stated that many oxygen compounds closely allied to the terpenes in chemical structure are found associated with them in plants. Cineol and terpineol have already been described, and here and there a reference has been made to substances having the formula  $C_{10}H_{16}O$ , and possessing the properties of ketones. Some of these, like the artificial products, dihydrocarvone or carvone, are unsaturated and have a mono-cyclic structure; others again, like natural fenchone and thujone, or carone which is prepared from carvone, are saturated and contain a bi-cyclic nucleus. The ketones are therefore divisible into two groups like the terpenes. In addition to the above are two important members of the camphor group, namely, *pulegone*, which belongs to the mono-cyclic division, and *camphor*, which must be included in the bi-cyclic division.

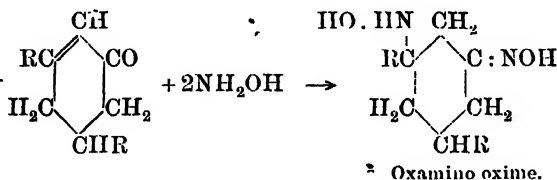
**Pulegone.** Pulegone is the chief constituent of oil of pennyroyal (*mentha pulegium*), and was isolated in 1891 by Beckmann and Pleissner, studied by Semmler and Wallach, and ultimately synthesised by Tiemann and Schmidt in 1896.<sup>1</sup> It is separated from the crude oil by means of the bisulphite compound and is dextro-rotatory. It forms an oxime and a semicarbazone, a crystalline hydrochloride and hydrobromide, and a characteristic nitroso compound having the double molecular formula  $(C_{10}H_{15}ONO)_2$ . Finally, on reduction with sodium and alcohol, it takes up two molecules of hydrogen and passes into ordinary *l*-menthol. These facts indicate an unsaturated ketone in which the ketone group is determined by its relation to menthol.<sup>2</sup>



<sup>1</sup> Ber., 1896, 29, 913; 1897, 30, 22.

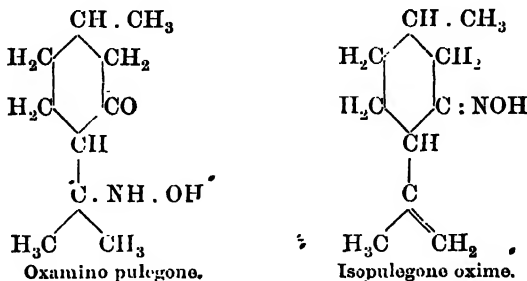
<sup>2</sup> The oxime is not, however, that of pulegone but of isomeric isopulegone, the action of the hydroxylamine having the effect of shifting the double bond. Wallach, *Annalen*, 1909, 365, 240.

The position of the double bond is also to some extent indicated by the behaviour of pulegone with hydroxylamine. It has been shown by Wallach and others that cyclic ketones with a double bond in the  $\alpha\beta$ -position to the ketone group exhibit a characteristic behaviour with hydroxylamine, depending upon whether the double bond is in the nucleus or side-chain. In the former case two molecules of hydroxylamine are utilized, and so-called *oxamino oximes* are formed in which one hydroxylamine molecule forms an additive compound at the double bond, and the other attaches itself to the ketone group.



In the second case, where the double bond is in the side-chain, either the additive or oxamino compound is formed, or else the true oxime.

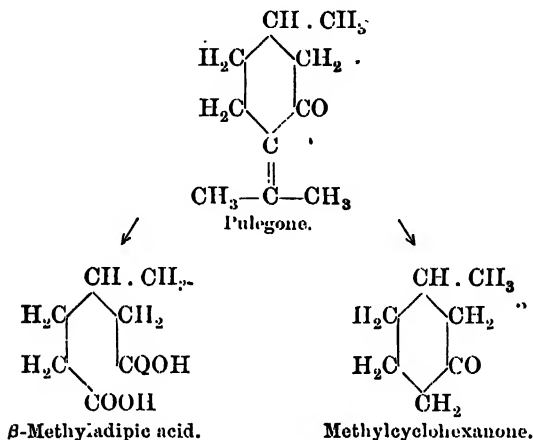
This is the case with pulegone, which forms both an oxime and an oxamino ketone. The double bond lies therefore in the isopropyl side-chain.



This conversion of pulegone into isopulegone is also effected by the

action of lead nitrate on pulegone hydrobromide, whilst the reverse change is accomplished by the action of baryta (p. 254).

This view of the structure of pulegone explains its oxidation by permanganate to acetone and  $\beta$ -methyladipic acid, and its decomposition on heating with formic acid or water to  $250^{\circ}$  into acetone and methyleyclohexanone.

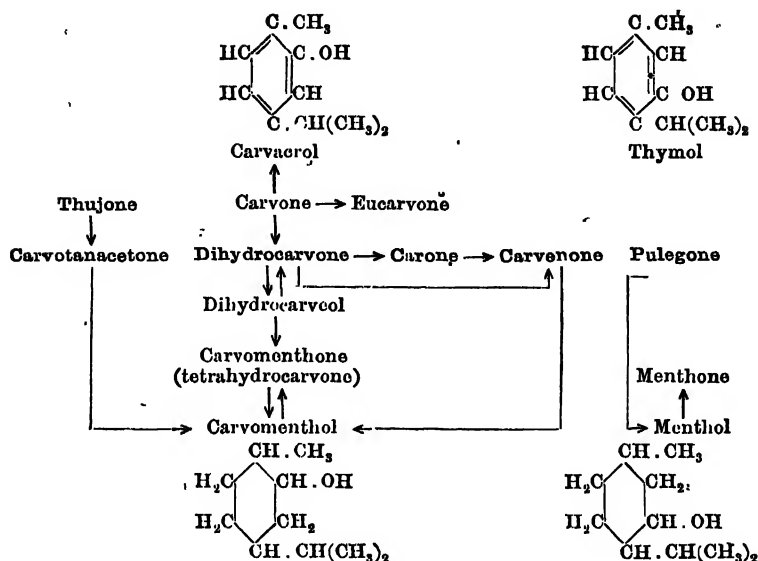
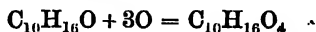


The interesting synthesis of pulegone from citronellal is described on p. 254.

The table on p. 234 shows the relationships existing between the mono-cyclic camphors and certain other compounds, which have been discussed in the foregoing section. The camphors are printed in thick type.

**Camphor.** Common or Japan camphor is one of the oldest known organic compounds. By reason of its crystalline character and easy purification, its extraordinary reactivity, its association with the terpenes, and its comparative abundance in nature, it has attracted the attention of more than one generation of chemists. It was analysed by Dumas and found to have the formula  $\text{C}_{10}\text{H}_{16}\text{O}$ . As early as 1785 its behaviour with nitric acid was studied by Kosegarten, who obtained the acid, now known as *camphoric acid*

the formation of which was afterwards correctly interpreted by Malaguti, Laurent and Liebig.



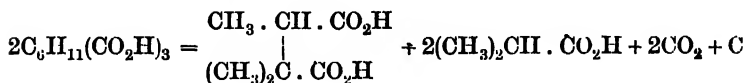
The Monocyclic Camphors and related Compounds.

During the last quarter of a century a host of skilful workers have concentrated their efforts in attempts at discovering its structure, with the result that no less than thirty different formulae have at one time or another been proposed. The earlier formulae were based on the behaviour of camphor with dehydrating and other agents. Phosphorus pentoxide or pentasulphide yield mainly *p*-cymene; zinc chloride produces a variety of aromatic hydrocarbons, among which toluene, *m*-cymene, *m*-xylene, *as*-ethyl-*o*-xylene, and tetramethyl benzene have been identified; hydriodic acid forms tetra- and hexa-hydro-*m*-xylene, and iodine produces considerable quantities of carvacrol (hydroxy-*p*-cymene).

The appearance of so many simple aromatic compounds clearly pointed to a benzene skeleton. It was only necessary to clothe it with suitable groups and side-chains, which should account for the ketonic nature of the compound, the formation of the dibasic camphoric acid, and as many of the aromatic hydrocarbons as their

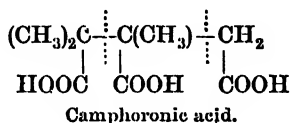
varied nature would admit of, in order to obtain a satisfactory formula. It is clear that the process admitted of varied treatment and some ingenuity. An entirely new light was thrown on the structure of camphor in 1893 by Bredt's<sup>1</sup> discovery of the constitution of camphoronic acid  $C_6H_{11}(CO_2H)_3$ , which Kachler<sup>2</sup> had found with camphoric acid among the oxidation products of camphor.

Bredt showed that when camphoronic acid is heated it breaks up into trimethylsuccinic acid and isobutyric acid, whilst carbon dioxide is evolved and some carbon is deposited. This decomposition may be expressed as follows:



Not only is the yield of trimethylsuccinic acid very considerable (60-70 per cent.), but its appearance cannot be ascribed to any secondary process, seeing that Koenigs obtained the same acid by the direct oxidation of camphoric acid with chromic acid.

Camphoronic acid, according to Bredt, is a trimethyltricarballic acid having the following formula:



Its conversion into trimethylsuccinic and isobutyric acid is readily explained by supposing the rupture to occur along one or other of the dotted lines. In the one case two molecules of isobutyric acid will be formed, and in the other, one molecule of trimethylsuccinic acid.

The structure of camphoronic acid has been completely established by Perkin, jun., and Thorpe,<sup>3</sup> who obtained it synthetically in the manner described in Part I, p. 219. The disposition of nine out of ten carbon atoms is thus accounted for, and as camphoronic acid is also obtained by oxidising camphoric acid, the two must contain the same grouping. Bredt showed that camphoronic acid is also formed when camphanic acid  $C_{10}H_{14}O_4$  is oxidised. Camphanic is a lactonic

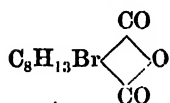
<sup>1</sup> Ber., 1893, 26, 3017; *Annalen*, 1896, 292, 55.

<sup>2</sup> *Annalen*, 1871, 159, 286.

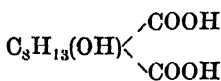
<sup>3</sup> *Trans. Chem. Soc.*, 1897, 71, 1169.



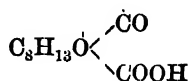
acid derived from hydroxycamphoric acid, and is produced when water or alkalis act upon bromocamphoric anhydride.



Bromocamphoric anhydride.

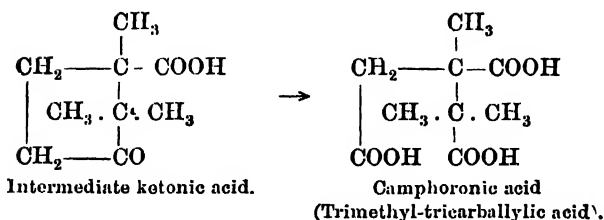
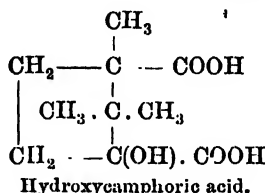
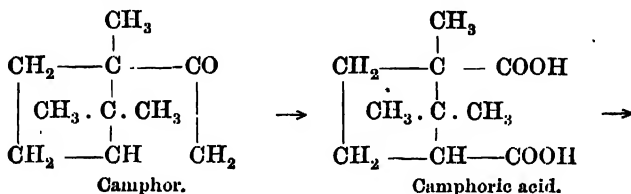


Intermediate product.



Camphanic acid.

According to Bredt the degradation of the camphor molecule on oxidation is represented as follows:

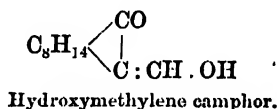
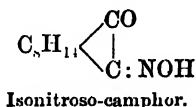


Bredt's formula was accepted with some reserve; for although it represented in a simple and natural fashion the stages in the resolution of the molecule by oxidation, it appeared to break down when submitted to the test of other reactions. Many of the difficulties at first encountered in adopting this formula have since been removed, a result to be mainly attributed to increased familiarity with the properties of 'ring', and more especially 'bridged-ring', structures. The occasion for balancing evidence in favour of one or other formula has fortunately disappeared; for Bredt's formula has received the best possible confirmation in the discovery of Komppa's

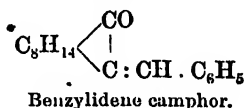
synthesis of camphoric acid. It only remains, therefore, to adjust Bredt's camphor formula to the facts and then to trace as briefly as possible the principal transformations which this many-sided compound undergoes.

1. *Camphor is a ketone*, for it forms an oxime,  $C_{10}H_{16}:NOH$ , a bromophenylhydrazone,  $C_{10}H_{16}:N.NH.C_6H_4Br$ , and a semicarbazone,  $C_{10}H_{16}:N.NHCONH_2$ . On reduction it yields the secondary alcohol, borneol,  $C_{10}H_{17}OH$ , and when heated with ammonium formate, the base bornylamine,  $C_{10}H_{17}NH_2$ , which is also formed when camphoroxime is reduced.

2. *Camphor contains the group*  $-CH_2.CO-$ . Claisen and Manasse prepared an isonitroso derivative and a hydroxymethylene camphor by the usual methods.

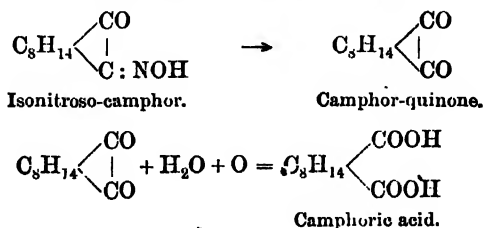


It also forms with benzaldehyde and its derivatives benzylidene compounds of the following type:



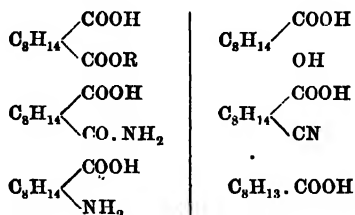
All these reactions are associated with the above  $-CH_2.CO-$  group.

3. *The group*  $-CH_2.CO-$  *is transformed into the two carboxyls of camphoric acid*. Claisen and Manasse showed that isonitroso-camphor, on hydrolysis, yields camphor-quinone, which, on oxidation, is converted easily and completely into camphoric acid.



Moreover, the two carboxyl groups are differently disposed, for there exist two series of acid esters (known as *ortho* and *allo* or  $\alpha$  and  $\beta$ ); two camphoramides, two amino acids, two hydroxy and two cyano acids, and also a corresponding series of four un-

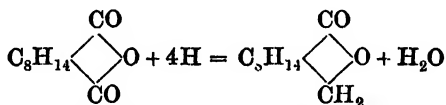
saturated monobasic acids,  $C_8H_{13} \cdot COOH$ , known as  $\alpha$ -campholytic  $\beta$ - or cis-campholytic (or isolaunolic) acid,  $\alpha\alpha$ -campholytic ( $\alpha$  launolic) acid, and an isomeric launolic acid, all of which will be described later (p. 246).



The *ortho* and *allo* ( $\alpha$  and  $\beta$ ) series of Camphoric acid derivatives.

The relation of camphoric acid to camphor is further established by the experiments of Haller,<sup>1</sup> who succeeded in transforming camphoric anhydride into camphor in the following way:

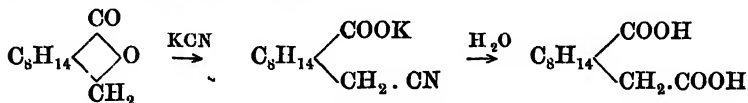
Camphoric anhydride can be reduced with sodium amalgam to campholide.



Camphoric anhydride.

Campholide.

Campholide, when heated with potassium cyanide, yields the nitrile of homocamphoric acid.

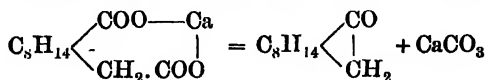


Campholide

Homocamphoric nitrile.

Homocamphoric acid.

Finally, the calcium salt is heated, and camphor distils.

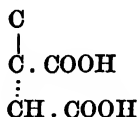


There are other ways of preparing homocamphoric acid directly from camphor, but they do not possess the theoretical interest which attaches to the synthesis from camphoric acid. It is a significant fact that whilst camphoric acid, like the succinic and glutaric acids, yields an anhydride on heating, homocamphoric acid does not, and consequently the carboxyls in the latter must be separated by at

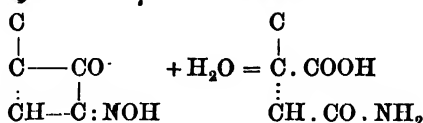
<sup>1</sup> *Compt. rend.*, 1896, 122, 446.

least a four-carbon chain. It follows that camphoric acid, which is the lower homologue, must possess a three-carbon chain or glutaric acid structure.

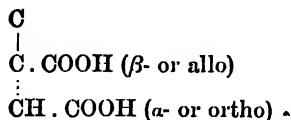
The structural relations between camphor and camphoric acid being thus clearly established, we have still to ascertain the cause which underlies the difference in the disposition of the two carboxyl groups, and to discover which of these two groups corresponds to the ketone and which to the methylene group in camphor. Chemical and physical evidence combine to prove that both camphor and camphoric acid are saturated compounds. By the action of bromine on camphoric anhydride substitution takes place, but only one monobromo derivative,  $\omega$ -bromocamphoric acid, is formed. As bromine always attaches itself to the  $\alpha$ -carbon in an acid, there can be but one  $\alpha$ -hydrogen, and camphoric acid will probably contain the group:



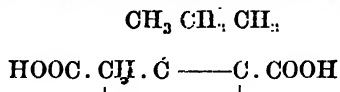
The differentiation of the  $\alpha$ - and  $\beta$ -series is arrived at in the following manner: when isonitroso-camphor is warmed with hydrochloric acid it is converted into camphoramidic acid of the  $\alpha$ -series.



It is the methylene group, therefore, which represents the  $\alpha$ -carboxyl in camphoric acid.

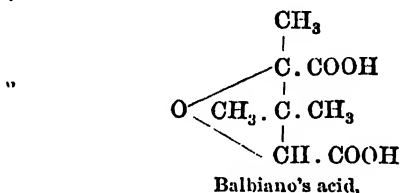


4. *Camphoric acid contains a trimethylglutaric group:*

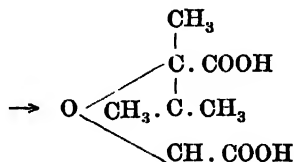
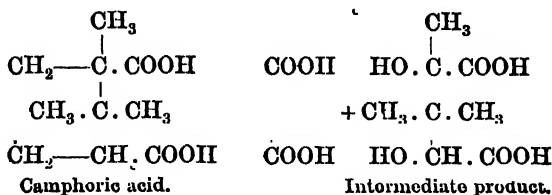


An important clue to the structure of camphoric acid was afforded by the researches of Balbiano. By oxidising camphoric acid with permanganate very slowly at the ordinary temperature, thereby

excluding the likelihood of intramolecular change, he obtained, in addition to small quantities of camphanic, camphoric, and trimethylsuccinic acids, a dibasic acid,  $C_8H_{12}O_5$ , and an equivalent quantity of oxalic acid as the chief products. This new acid, on reduction with hydriodic acid, gave, among other products, an acid,  $C_7H_{14}O_4$ , which was identified as  $\alpha\beta$ -trimethylglutaric acid, afterwards prepared synthetically by Perkin and Thorpe.<sup>1</sup> Balbiano's acid does not possess ketonic properties, for, although it combines with hydroxylamine and hydrazines, the products are of the nature of additive compounds, and the compound almost certainly represents the inner ether of a dihydroxytrimethylglutaric acid, having the structure,



Its appearance in company with oxalic acid is very simply explained by the aid of Breté's formula:

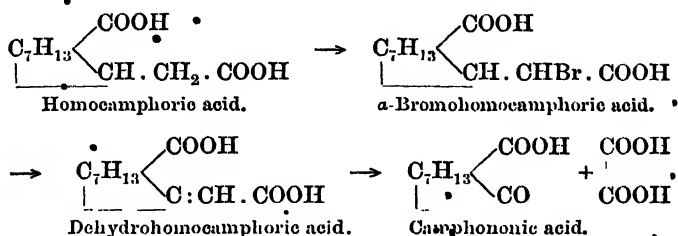


5. *Camphoric acid is a derivative of Cyclopentane.* The presence of a 5-carbon ring in camphoric acid appears very probable when the following evidence is considered. Lapworth<sup>2</sup> showed that when homocamphoric acid is brominated and hydrobromic acid then

<sup>1</sup> *Trans. Chem. Soc.*, 1899, 75, 61.

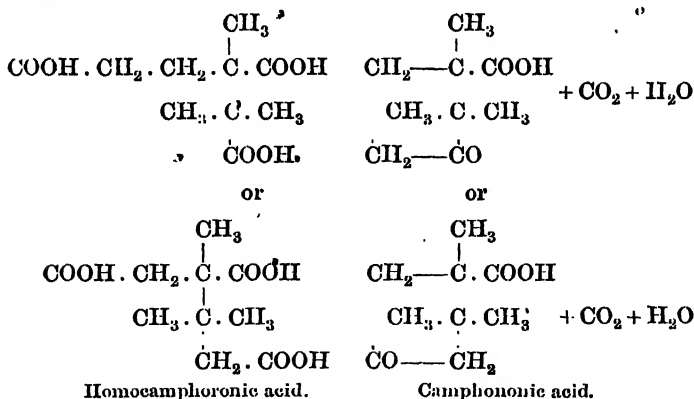
<sup>2</sup> *Trans. Chem. Soc.*, 1900, 77, 1053.

removed, an unsaturated dehydrohomocamphoric acid is formed which yields oxalic and camphononic acid on oxidation.

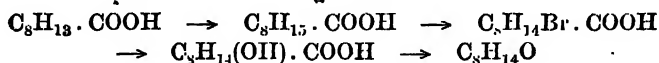


Now camphononic acid is a saturated kotic acid which may also be obtained by heating the anhydride of homocamphoric acid to 200–260°.

Homocamphoric acid, which is probably represented by one of the following structural formulae, yields camphononic acid as follows:

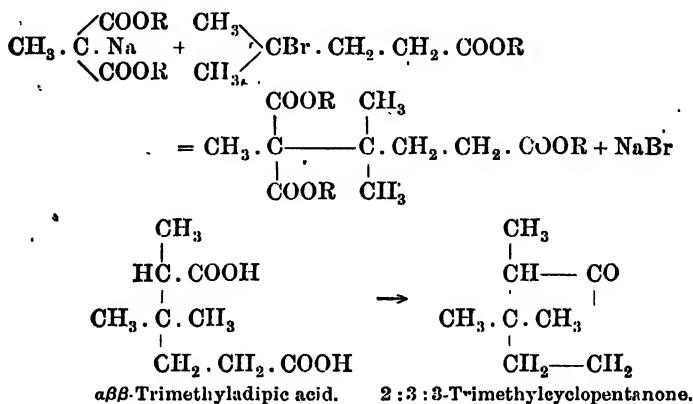


Another piece of evidence of a similar nature has been contributed by Noyes.<sup>1</sup> Isolauroic acid (see p. 218) gives on reduction a dihydro-derivative in which one  $\alpha$ -hydrogen can be replaced by bromine. By the action of baryta on the bromine compound a hydroxy-dihydro-isolauroic acid is obtained which, on oxidation, loses carbon dioxide and forms a ketone.

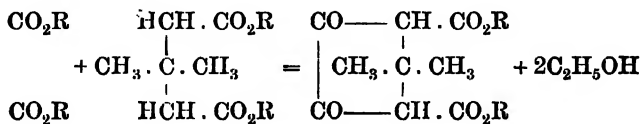


<sup>1</sup> Ber., 1899, 32, 2288

This ketone was synthesized and identified as 2:3:3-trimethyl cyclopentanone in the following way: by combining sodium methyl malonic ester with  $\gamma$ -bromoisocaproic ester, the ester of a tribasic acid was obtained which, on hydrolysis, gave the acid. This acid loses carbon dioxide on heating, and passes into  $\alpha\beta$ -trimethyladipic acid, the lime salt of which is converted by heat into the ketone in question.

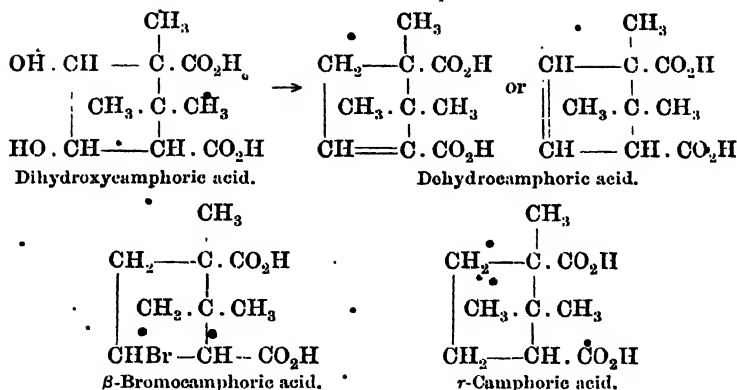


6. *Komppa's synthesis of camphoric acid.* This synthesis affords the most convincing proof of the correctness of Brecht's formula. Ethyl-diketoapocamphorate, which is the starting-point, was prepared by Komppa<sup>1</sup> by condensing ethyl oxalate with ethyl  $\beta\beta$ -dimethylglutarate (Part I, p. 227).



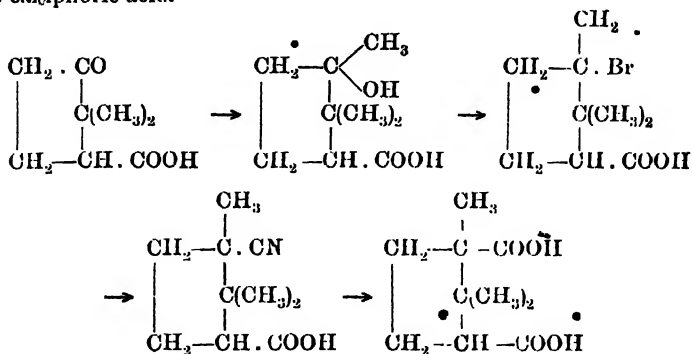
A methyl group was then introduced by the action of sodium and methyl iodide. The product was reduced to dihydroxycamphoric acid, and then boiled with hydriodic acid and red phosphorus and converted into the unsaturated acid, dehydrocamphoric acid. The latter combines with hydrobromic acid, and forms a  $\beta$ -bromocamphoric acid, and is then reduced with zinc dust and acetic acid to *r*-camphoric acid, which is identical with the racemic product obtained from camphor by oxidation.

<sup>1</sup> Ber., 1901, 34, 2472; 1903, 36, 4332.



By a similar process Komppa has prepared the homologue of camphoric acid by introducing an ethyl in place of a methyl radical.<sup>1</sup>

Perkin and Thorpe<sup>2</sup> have also succeeded in synthesising camphoric acid from dimethyl cyclopentanone carboxylic acid (prepared from dimethylbutane tricarboxylic acid). This acid in the form of its ester reacts with magnesium methyl iodide and gives the alcohol, which is then converted into the bromide with hydrobromic acid. The latter reacts with hydrogen cyanide and potassium cyanide, and gives a cyanide which on hydrolysis is transformed into *dl*-camphoric acid.



The inactive acid has since been resolved into its active components by crystallisation of its cinchonidine salt.<sup>3</sup>

<sup>1</sup> Ber., 1911, 44, 858.

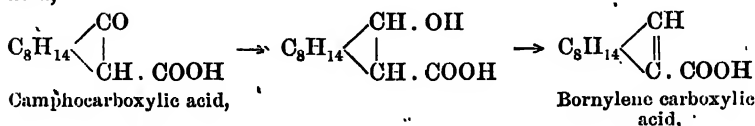
<sup>2</sup> Trans. Chem. Soc., 1906, 89, 795.

<sup>3</sup> Beckmann, Ber., 1909, 42, 185.

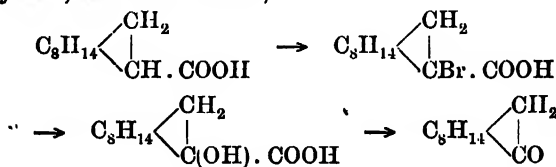


The synthesis of camphor is therefore complete, since camphoric acid can be converted into camphor by the method described on p. 238.

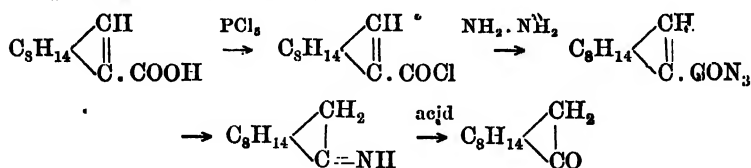
An isomeric  $\beta$ - or  $\epsilon$ -camphor, in which the positions of the CO and  $\text{CH}_2$  groups are reversed, has been synthesised by Perkin and Lank-shear<sup>1</sup> and by Brecht and Hilbing.<sup>2</sup> Both sets of observers start from bornylene carboxylic acid, which is obtained from camphocarboxylic acid,



but whilst the former reduce, brominate and convert it into the hydroxy acid, and then oxidise,



the latter convert it into the acid chloride, the azide, the amino camphor, and finally  $\beta$ -camphor.



Having now reviewed the principal evidence in favour of Brecht's formula for camphor, we will proceed to criticize it in the light of some of its less salient but not less important features.

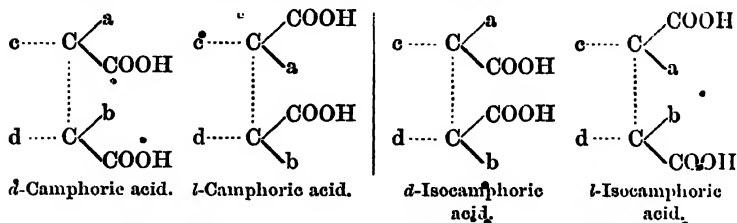
Both camphor and camphoric acid are optically active. Camphor is found in two-nantiomorphous forms, Japan camphor being dextro-rotatory, and Matricaria camphor laevo-rotatory; camphoric acid has been obtained in four active modifications, a *d*- and *l*-camphoric acid and a *d*- and *l*-isocamphoric acid,<sup>3</sup> and two racemic compounds derived from them. The *d*- and *l*-camphoric acids are obtained from the two active camphors by oxidation, and each can be converted into the corresponding isocamphoric compound. The formula for camphoric acid harmonizes with the existence of two pairs of active

<sup>1</sup> Proc. Chem. Soc., 1911, 27, 166.

<sup>2</sup> Chem. Centralbl., 1911, II, 954.

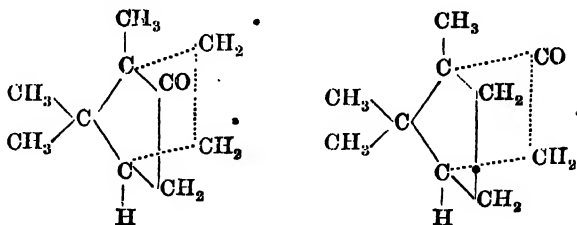
<sup>3</sup> Aschan, Ber., 1894, 27, 2001; *Annalen*, 1901, 316, 196.

enantiomorphs, for it contains two asymmetric carbon atoms. The four active compounds may be represented as follows:



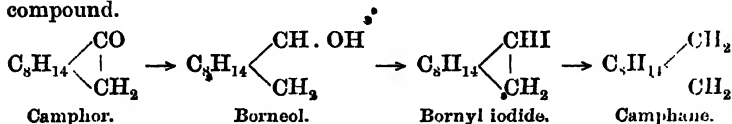
The first pair have a *cis*, the second a *trans* configuration, and in accordance with the theory the former yield anhydrides, whereas the latter do not.

The reason for the existence of only two active camphors is not so obvious, for camphor contains the same two asymmetric carbon atoms as camphoric acid. There is, however, this difference, that the two carbon atoms, which in camphoric acid are represented by carboxyls and are free to assume independent positions, are linked together in camphor. It follows that there are only two active camphors which correspond to *d*- and *l*-camphoric acid, and may be represented in the following manner:



*d*- and *l*-Camphor

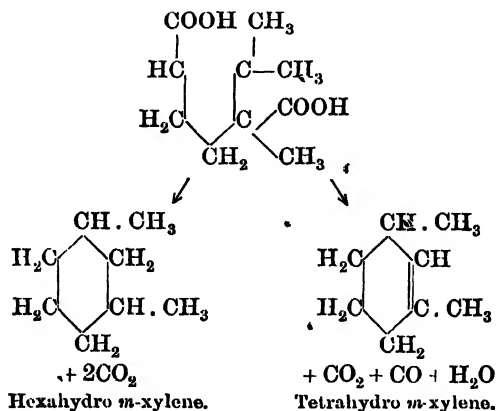
It will at once be obvious that the asymmetry of the camphor molecule as represented above depends on the presence of the ketone group. That this is the case has been demonstrated by Aschan,<sup>1</sup> who succeeded in converting the ketone group by reduction into a methylene group in the manner indicated below, with the object of producing a completely symmetrical and consequently inactive compound.



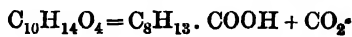
<sup>1</sup> *Aschan*, 1901, 316, 229.

The reduction was carried out in such a way as to preclude racemisation, with the result that the hydrocarbon camphane was quite inactive.

Some of the opposition which Bredt's formula at one time encountered was due to the difficulty of reconciling it, with the production of substances like *m*-xylene and its tetra- and hexahydro-derivatives. The formation of the various benzene hydrocarbons, though it must remain more or less a matter of conjecture owing to the difficulty of following the changes in detail, is readily explained, since it is now a well-established fact that under certain conditions the methyl side-chain of a cyclopentane derivative may become fused into the ring of a cyclohexane compound. The conversion of camphoric acid into derivatives of *m*-xylene may very well take place in accordance with the following scheme :

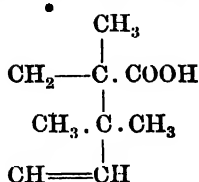


But the real stumbling-block which has stood longest in the way of the Bredt formula has been the intractable nature of isolauronic and  $\beta$ -campholenic acids. In order to understand the position occupied by these acids it is necessary to consider them in conjunction with their isomers from the point of view of their preparation, as well as of their properties. An acid of the formula C<sub>8</sub>H<sub>13</sub>·COOH, called lauronic acid, was first observed by Fittig and Wofinger.<sup>1</sup> It is obtained by the action of water or alkalis on bromocamphoric anhydride, or by the dry distillation of camphanic acid (see p. 236).

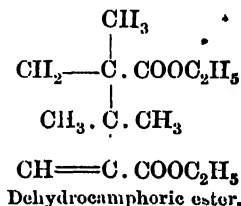
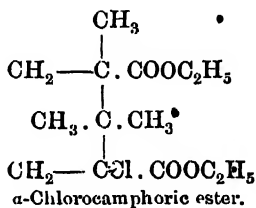


<sup>1</sup> *Annalen*, 1885, 327, 1.

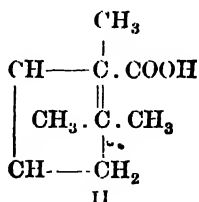
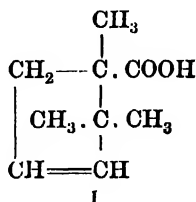
These reactions appear to point to the following formula :



But this acid should give camphoronic acid on oxidation (p. 235), which it does not, but on the contrary forms a variety of other products, quite irreconcilable with the above structure.<sup>1</sup> A second lauronolic acid was obtained by Bredt from  $\alpha$ -chlorocamphoric ester, which on treatment with quinoline loses hydrogen chloride, and gives the ester of an unsaturated dibasic acid (dehydrocamphoric acid), from which carbon dioxide can be removed on heating and lauronolic acid generated.



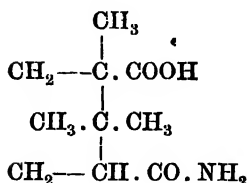
As Bredt's acid gives camphoronic acid on oxidation it must have the structure originally assigned to the previous compound I, whilst Fittig and Woringen's acid will probably be represented by II.<sup>2</sup>



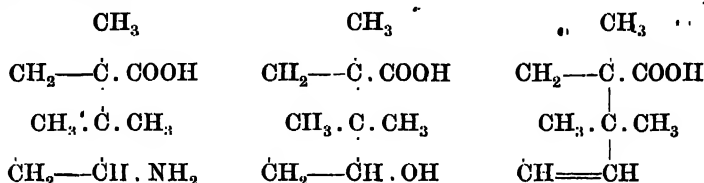
The structure of Bredt's acid is also in agreement with its formation from  $\alpha$ -camphoramic acid. The latter is obtained by the action of ammonia on camphoric anhydride, and its constitution is determined by its production from isonitrosocamphor (see p. 239).

*Ber.*, 1900, 33, 2949.

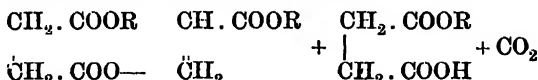
*Bredt, J. prakt. Chem.*, 1911, 83, 400.



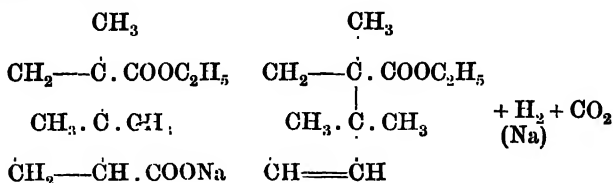
From it Noyes obtained by the action of sodium hypobromite the amino acid, which nitrous acid converts partly into the hydroxy-derivative, partly, by elimination of a molecule of water, into lauronic acid.



The same acid has also been obtained by Walker<sup>1</sup> by the electrolysis of the sodium salt of the allo ethyl ester of camphoric acid (see p. 238). According to the usual behaviour of dibasic acid esters under the influence of the current, carbon dioxide is split off, and together with certain other products an unsaturated acid is formed. Ethyl sodium succinate gives acrylic ester.



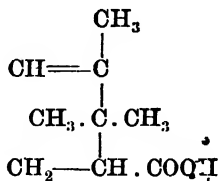
The formation of lauronic acid takes place in accordance with a similar scheme:



By the same process Walker converted the sodium salt of the ortho ethyl ester into a mixture of  $\alpha$ -campholytic and isolauronic (*cis*-campholytic) acids, and Noyes obtained the same two acids from  $\beta$ -camphoramidic acid by repeating the same series of changes which produced lauronic acid from the  $\alpha$ -compound. As isolauronic acid

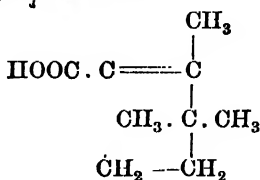
<sup>1</sup> *Trans. Chem. Soc.*, 1893, 63, 495; 1895, 67, 337.

is easily obtained from the  $\alpha$ -compound by boiling with dilute sulphuric acid, they have been regarded as stereoisomeric; but this is doubtful. Assuming it to be the case, the natural inference would be that both acids possess the formula,



but here a difficulty arises, for the isolaunolic acid is undoubtedly an  $\alpha\beta$ -unsaturated acid. It is inactive, which would scarcely be the case if the above formula held, seeing that it contains one of the original asymmetric carbon atoms of camphoric acid undisturbed. and it cannot be resolved into active constituents; it forms a dibromide from which alkalis remove not only hydrobromic acid, but also carbon dioxide, a change which Fittig's researches have shown to be characteristic of  $\alpha\beta$ -unsaturated acids. Furthermore, dihydro isolaunolic acid, obtained from the original acid by reduction, gives on bromination a monobromo derivative, from which alkalis remove hydrogen bromide and regenerate isolaunolic acid. As the bromine substitutes hydrogen in the  $\alpha$ -position to the carboxyl, the double link must necessarily occupy the  $\alpha\beta$ -position.

The synthesis of isolaunolic acid by Perkin and Thorpe<sup>1</sup> has fixed the structure beyond all doubt.



Isolaunolic acid.

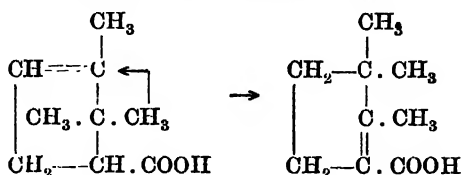
But how is such a structure to be reconciled with its formation from  $\beta$ -camphoramie and ortho camphoric ester? A very simple explanation has been suggested by Lapworth<sup>2</sup> and adopted by Blaise and Blanc<sup>3</sup> which is based upon a change similar to the pinacol-pinacone conversion (Part II, p. 357). Supposing in the above structure one of the 1:1-methyl groups passed to the adjoining

<sup>1</sup> *Trans.*, 1904, 85, 128.

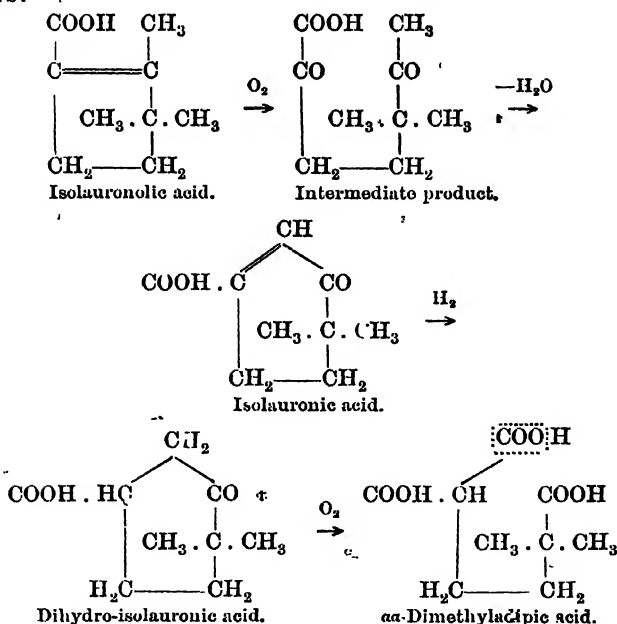
<sup>2</sup> *B. A. Reports*, 1900, 325.

<sup>3</sup> *Bull. Soc. Chim.*, 1900 (3), 23, 167.

carbon with a corresponding readjustment of the double bond, an acid of the required formula would be produced.



The difficulty involved in dealing with such mobile complexes as camphor and its degradation products is further illustrated by the behaviour of isolauronic acid on oxidation. According to Koenigs and Meyer<sup>1</sup> it yields isolauronic acid,  $\text{C}_9\text{H}_{12}\text{O}_3$ , which Perkin, jun.,<sup>2</sup> characterized as a ketonic acid, and Blanc<sup>3</sup> identified as a cyclohexane derivative. Blanc's view was arrived at from the observation that isolauronic acid on reduction forms a dihydro derivative,  $\text{C}_9\text{H}_{14}\text{O}_3$ , which on heating with sodium hypobromite loses carbon dioxide and gives *aa*-dimethyladipic acid. These changes are explained as follows:



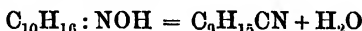
<sup>1</sup> Ber., 1894, 27, 3466.

<sup>2</sup> Trans. Chem. Soc., 1898, 73, 802.

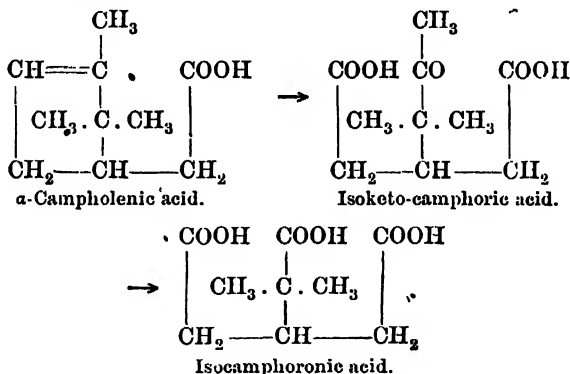
<sup>3</sup> Compt. rend., 1900, 130, 849.

It is only another case of the convertibility of cyclopentane and cyclohexane structures (see p. 246).

The theory of the formation of isolaunonic acid has served to explain the structure of  $\beta$ -campholenic acid. There are two,  $\alpha$ - and  $\beta$ -campholenic acids. They can be obtained in a variety of ways, as, for example, by the dehydration of camphoroxime, which yields the nitriles of the two acids in question.



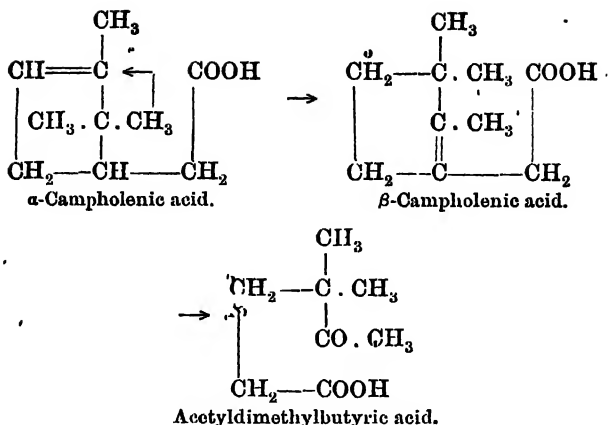
Like  $\alpha$ -campholytic acid,  $\alpha$ -campholenic acid can be converted by the aid of different reagents into the  $\beta$ -compound, which is probably a secondary product of the above reaction. The structure of the  $\alpha$ -compound offers no difficulty. It is dextro-rotatory, and contains, therefore, an asymmetric carbon; it gives, on oxidation, successively a dihydroxy derivative, isoketo-camphoric acid, and finally isocamphoronic acid, the structure of which has been ascertained by synthesis (Part I, p. 203).<sup>1</sup> All these changes are expressed simply and naturally as follows:



The  $\beta$ -acid, on the other hand, is inactive, and yields an inactive dihydroxy derivative on oxidation, which subsequently breaks up into oxalic acid and  $\gamma$ -acetyldimethylbutyric acid. The appearance of these two acids in conjunction with a structure which contains no asymmetric carbon is at first a little perplexing; but the observation that isolaunonic acid gives the same  $\gamma$ -acetyldimethylbutyric acid has suggested the homology of the two acids and the probability of a similar shifting of a methyl group in the formation of the  $\beta$ - from the  $\alpha$ -compound.

<sup>1</sup> Perkin, jun., *Trans. Chem. Soc.*, 1899, 75, 897.





That the above structure represents the true formula of the  $\beta$ -acid has been proved by the synthesis of  $\beta$ -campholenic lactone by Blanc.<sup>1</sup>

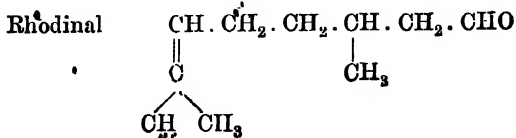
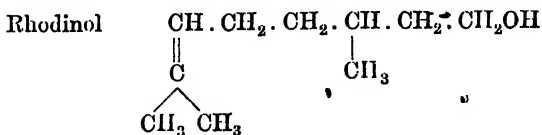
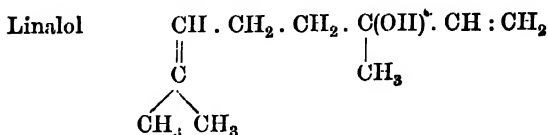
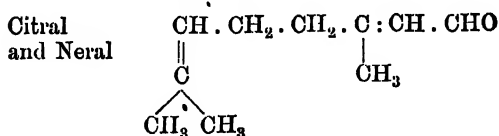
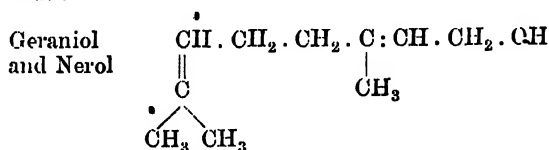
Our account of camphor must draw to a close. The manifold changes of this mobile molecule—a veritable Proteus among organic compounds—are far from being exhausted, but the reference books must be consulted for further information. Our object in the foregoing account has merely been to give the reader some idea of the transformations which camphor and its products undergo under different conditions, and to throw some light on the difficulties which have attended the inquiry into its structural formula. The discovery of the constitution of camphor has now been accomplished, and the experience thus gained has vastly increased our knowledge of cyclic structures.

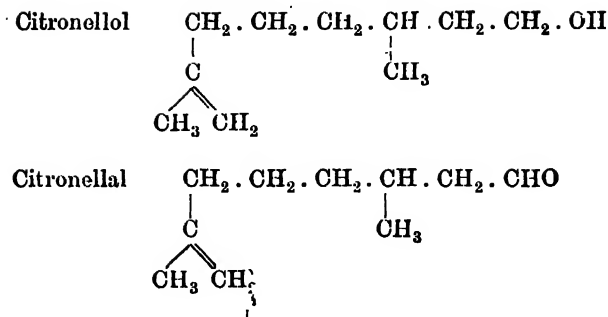
The relation of pine $\acute{e}$ ne to camphor has been turned to profitable account by the elaboration of an industrial process for the manufacture of camphor from turpentine. There are various modifications of the method in the matter of detail, but it consists essentially in the successive formation of camphene hydrochloride, camphene (isobornyl ester), isoborneol, and camphor (see p. 220).

**The Olefinic Terpenes and Camphors.** The constituents of the essential oils which have been described in the previous section belong to saturated or unsaturated cyclic systems; but in recent years a new group of open-chain compounds of the formula  $\text{C}_{10}\text{H}_{16}$ ,  $\text{C}_{10}\text{H}_{16}\text{O}$ , and  $\text{C}_{10}\text{H}_{18}\text{O}$  has been discovered and described by Tiemann and Semmler, and named by them *olefinic terpenes and*

*camphors.* The name does not merely indicate similarity in composition with the terpenes and camphors, but a very close relationship in chemical structure. Moreover, they have been recognized as responsible in a great measure for the delicate aroma of those essential oils in which they occur. The perfume of orange-blossom, rose, and lavender is due to the presence of minute quantities of these substances. They exhibit among themselves a certain similarity in structure and, at the same time, show an unmistakable connection with the terpene and camphor group. They contain ten carbon atoms, which are disposed in such a way that six form a straight chain, three of them form an unsaturated isopropyl group attached to one end of the chain, and the tenth a methyl group at the fourth carbon atom from the end of the chain. In other words, the grouping may be conceived to resemble that of a monocyclic terpene or camphor in which the ring has been ruptured.

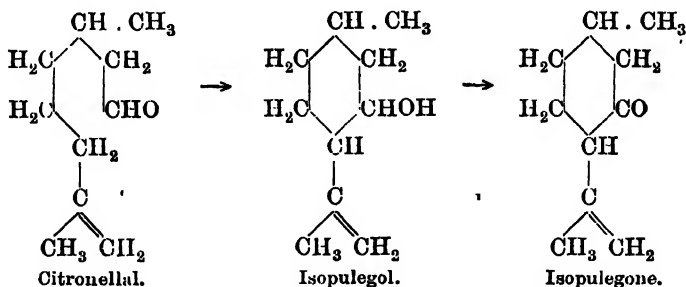
The following is the probable structure of some of these compounds :



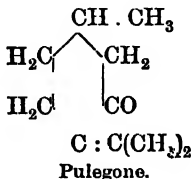


This conception is not a purely theoretical one, for we shall see presently that cyclic terpenes can be prepared by simple methods from these compounds, and, by a reversal of the process, certain cyclic compounds may be converted into open-chain members of the group.

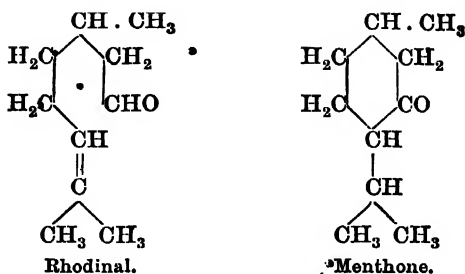
Thus, citronellal when heated with acetic anhydride changes into isopulegol, and on oxidation into isopulegone,



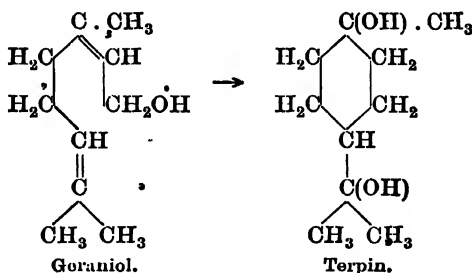
and the latter, by the action of baryta, undergoes isomeric change to pulegone,



whereas the isomeric rhodinal under the same conditions isomerises to menthone.

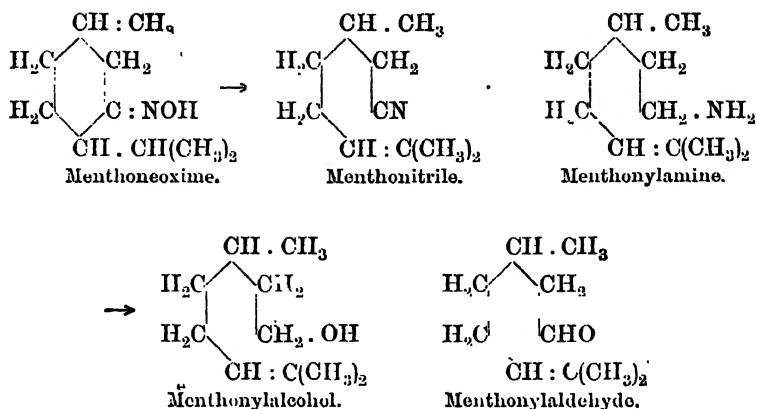


Linalol in presence of formic acid at  $30^\circ$  is converted into a mixture of dipentene and terpinene.<sup>1</sup> This change is doubtless brought about in the first instance by the isomeric change of linalol to geraniol, which acids are known to effect. By the removal and subsequent addition of the elements of water, ring formation occurs with the production of terpin, followed by that of dipentene and terpinene.



An example of formation of an open-chain structure from a cyclic ketone is presented by menthone, which by the following series of operations yields a product isomeric and possibly identical with citronellal and having the perfume of roses. The ketone is converted into the oxime, which by dehydration gives menthonitrile. The latter is reduced in the ordinary way to menthonylamine, which is then decomposed by nitrous acid. The alcohol, thus formed, is oxidised to the aldehyde.

<sup>1</sup> *J. prakt. Chem.*, 1892 (2), 45, 601.



The importance of the olefinic terpenes and camphors from an industrial point of view cannot be estimated too highly; for the aroma of many perfumes is undoubtedly due to their presence. Their scientific study, which is intimately linked with the industry of the essential oils, has prepared the way for new developments in synthetic chemistry.

The following is a brief description of the more important members of this group of compounds.

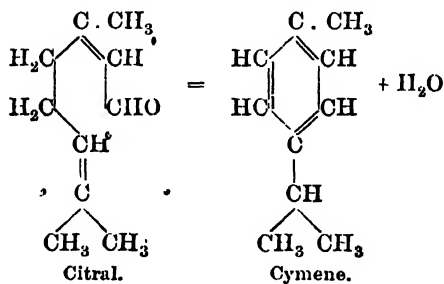
**Geraniol.** In 1890 Semmler showed that the alcohol isolated by Jacobsen<sup>1</sup> in 1871 from Indian geranium oil (by means of the solid compound which it forms with calcium chloride), and having the formula  $\text{C}_{10}\text{H}_{18}\text{O}$ , was not a cyclic compound; but by reason of the additive compound which it forms with bromine, and its constant of refraction, must contain two double bonds, and was in all probability an unsaturated open-chain compound. Geraniol is widely diffused, being found in German and Turkish rose oil, in citronella and lemon-grass oils, and in smaller quantities in ylang-ylang, lavender, and other essential oils. On careful oxidation with chromic acid mixture it is converted into an aldehyde, citral, a substance which is also found in nature in different kinds of lemon oil.<sup>2</sup> As geraniol can be obtained from citral by reduction, and as citral has been prepared artificially (see below), geraniol must also be ranked among synthetic products. It is further obtained from linalol, which undergoes isomeric change on heating with acetic anhydride, and, conversely, geraniol can

<sup>1</sup> *Annalen*, 1871, 157, 232.

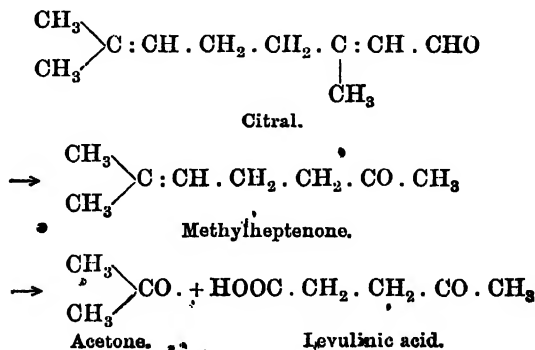
<sup>2</sup> Semmler, *Ber.*, 1891, 24, 201.

be transformed into linalol by heating with water to 200°, whilst certain dehydrating agents, like potassium hydrogen sulphate, convert geraniol into the olefinic terpene *geraniene*,  $C_{10}H_{16}$ ; formic acid produces dipentene and terpinene, and a mixture of acetic and sulphuric acid forms terpineol (m. p. 35°). The structure of geraniol depends upon that of citral.

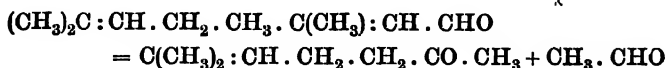
*Citral* (*geraniol*) is a common constituent of essential oils, and it is to this substance that lemon oil owes its delicate aroma. It is very abundant in lemon-grass oil (70–80 per cent.), and is an important constituent of orange, mandarin, lime, and certain kinds of eucalyptus oil. It is a di-olefinic aldehyde, for it gives the usual reactions for aldehydes, including its conversion into *geranic acid* on oxidation, and it also unites with two molecules of bromine. It is very sensitive to acid reagents, and by the action of dilute sulphuric acid and potassium bisulphate it loses water and passes into cymene.



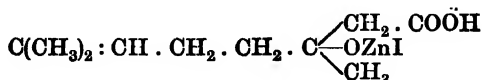
Its structure is derived from its behaviour with chromic acid mixture, which breaks it up into methylheptenone in the first instance, and finally into acetone and levulinic acid,



and also from its conversion into methylheptenone and acetaldehyde by the action of potassium carbonate.



The syntheses of citral and geranic acid which confirm the above formula have been effected by Barbier and Bouveault<sup>1</sup> from methylheptenone in the following way: the latter reacts with zinc and iodacetic acid (Part I, p. 218), giving the compound—



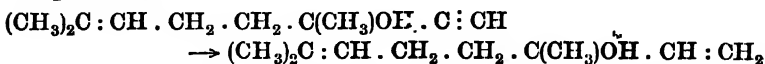
which is converted on the addition of dilute acid into the hydroxy acid—



losing water and yielding geranic acid on distillation with acetic anhydride. By distilling the calcium salt of geranic acid with calcium formate Tiemann<sup>2</sup> obtained citral.

*Linalol* is widely distributed, and occurs in optically active forms. It is dextrogyrate in coriander oil, but in no other oil, whereas the laevo compound is found partly free and partly as linalyl acetate in the oils of linaloes, bergamot, neroli, petitgrain, limette, spike, lavender, sage, thyme, spearmint, origanum, ylang-ylang, &c. It readily undergoes change with acids; organic acids convert it into the isomeric geraniol, whilst small quantities of sulphuric acid produce terpineol, and by shaking with 5 per cent. sulphuric acid terpin hydrate is formed. Its conversion into dipentene and terpinene has already been described (p. 255).

It has been synthesized by condensing methylheptenone and acetylene in presence of iodamide and subsequent reduction of the product.<sup>3</sup>



*Rhodinol* is found in geranium and rose oil, in the laevo form, whilst the inactive compound was obtained by the reduction of the ester of geranic acid.

<sup>1</sup> *Compt. rend.*, 1896, 122, 393.

<sup>2</sup> *Ber.*, 1898, 31, 827.

<sup>3</sup> Ruzicka and Fornasir, *Abstr.*, 1919, i, 198.

*Rhodinal* was obtained by Barbier and Bouveault<sup>1</sup> by oxidation of rhodinol, and differs from citronellal, with which it is isomeric, by passing into menthone, which gives a characteristic semicarbazone. When further oxidised, rhodinal forms rhodinic acid.

*Citronellol* contains two atoms of hydrogen more than geraniol and linalol. It is found in geranium oils in both active forms, the laevogyrate predominating. Mixed with geraniol it is present in various rose oils. It can also be prepared artificially from the aldehyde citronellal by reduction.

*Citronellal*, the chief constituent of citronella oil (from *Andropogon nardus*) and oil of *Eucalyptus maculata*, is frequently found accompanying citral in lemon and rose oil, from which, however, it is easily distinguished by its optical activity. Its structure is determined by oxidation, which breaks it up into acetone and  $\beta$ -methyladipic acid. It is an interesting fact that acetic anhydride converts citronellal into the isomeric isopulegol, which can be transformed successively into isopulegone and the natural pulegone (p. 231).

*Nerol*, which is found in neroli and petitgrain oil, and its oxidation product, *neral*, are probably stereoisomeric with geraniol and citral.<sup>2</sup>

**Natural and Artificial Perfumes.** The history of perfumes and essences carries us back to very remote times, but the scientific study of the chemical nature of the substances which afford the aroma is of comparatively recent date, and may be said to have begun with the researches of Wallach and Tiemann a quarter of a century ago. The results of some of these investigations have been presented in the foregoing pages, in which the properties of the terpenes and camphors and their olefinic analogues are described, but the list of aroma-bearing constituents of plants is by no means complete. Some of the simpler aromatic compounds, like oil of bitter almonds (benzaldehyde), oil of wintergreen (methyl salicylate), methyl anthranilate, thymol, carvacrol, eugenol, vanillin, coumarin, safrole, anethole, and many other natural perfumes have not been included, and little, if anything, has been said on the subject of their artificial preparation. The knowledge that a peculiar perfume had its origin in a definite chemical individual which could be isolated in a pure and therefore concentrated form, the development of analytical processes which effected the separation of these constituents, and finally the attempts which in many cases were carried to a successful issue, of producing

<sup>1</sup> *Compt. rend.*, 1896, 122, 737.

<sup>2</sup> *Zeitsch. chem.*, 1906, 38, 1780.

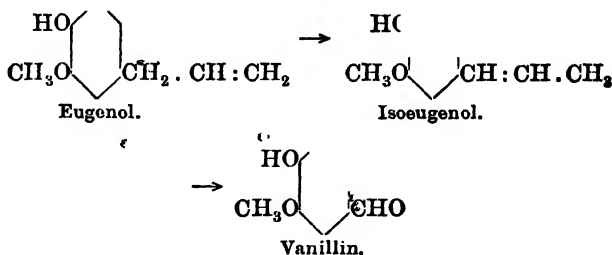


the aroma-bearing substance artificially, have given an extraordinary impetus to the development of the perfume industry, especially by chemical manufacturers in Germany. The history of this development is only another instance of the successful application of pure science to technology, of which the artificial colour industry is so striking an example.

We do not propose to do more than to give a brief account of some of the more important natural and artificial perfumes not included among the substances already described. Shortly after Mansfield's discovery of the production of nitrobenzene from benzene in 1847, Collas introduced it as 'essence of mirbane' into commerce. A few years later the esters of the fatty acids appeared as apple, pine-apple, pear essences, &c. In 1844 Cahours found that methyl salicylate was the chief constituent of wintergreen oil, and this discovery soon led to its artificial preparation from synthetic salicylic acid.

In 1868 Cahours found a process for preparing benzaldehyde from benzal chloride, and this discovery was the forerunner of other artificial aldehydes with characteristic scents; in 1875 Perkin obtained coumarin synthetically (Part I, p. 248); in 1888 artificial musk (trinitro-*ψ*-butyltoluene) was discovered by Baur, and other strongly scented di- and tri-nitro compounds have since appeared.

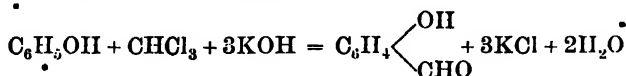
The first synthesis of vanillin, the sweet-smelling constituent of the vanilla pod, of which it constitutes about 1 per cent., was accomplished by Tiemann and Haarmann in 1875. It was obtained by oxidising coniferyl alcohol, a constituent of the glucoside, coniferin (p. 41). In the following year coniferyl alcohol was replaced by eugenol, which is present to the extent of 70–90 per cent. in oil of cloves. Eugenol is first converted by boiling amyl alcoholic potash into isoeugenol,<sup>1</sup> which is then oxidised to vanillin.



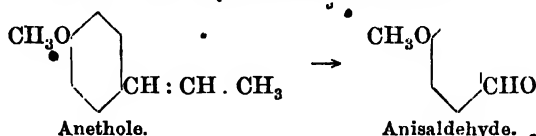
Since then a dozen different methods have been devised for preparing this substance, and are described in books of reference.

<sup>1</sup> Ciamician and Silber, *Ber.*, 1890, 23, 1160.

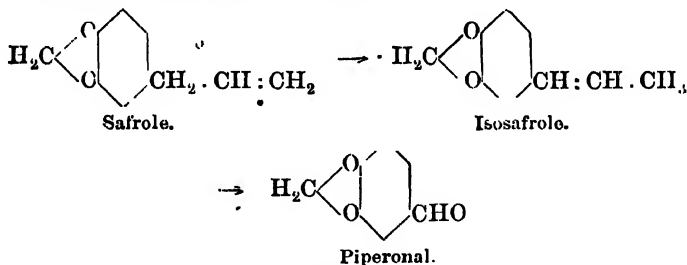
Of the many artificial aldehydes which are used as perfumes the following may be mentioned: *cuminaldehyde* gives the odour to cumin oil; *salicylaldehyde* is present in spiraea oil, and is obtained artificially by the action of chloroform and potash on phenol by Reimer's reaction, and by other methods.



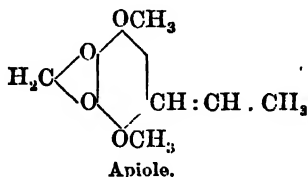
*Anisaldehyde* is prepared by the oxidation of anethole, the chief constituent of anise oil, and has the odour of hawthorn.



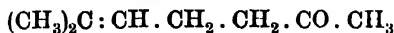
*Cinnamic aldehyde* or cinnamol is the chief constituent of cinnamon and cassia oil (75-90 per cent.); *piperonal* or heliotropin is obtained from safrole, which is first converted into isosafrole and then oxidised, and possesses the scent of heliotrope.



*Apiole* is an ether related to safrole, and is the aromatic constituent of parsley seed oil.



The aliphatic ketones which are found in nature, and have been prepared artificially, include *methylheptenone*.



which smells like amyl acetate and methyl nonyl ketone,  $\text{CH}_3 \cdot \text{CO} \cdot \text{C}_8\text{H}_{17}$ , which imparts the scent to oil of rue.

The natural cyclic ketones play a very important rôle as perfumes, and many of them have already been described. The most interesting is *ionone*, the natural perfume of the violet, which has been so closely imitated in the form of ionone by Tiemann, both in structure and scent, as to rank among the crowning achievements in organic synthesis (Part I, p. 240).

*Chavicol* and *estragol* are related to anethole, and accompany it in aniseed and other oils.

Among recent discoveries in the chemistry of perfumes is that of methyl anthranilate and indole, both of which impart their aroma to jasmine, whilst methyl anthranilate is also present in neroli, lemon and several other essential oils.

The following table contains some typical examples of essential oils and their chief constituents. The less important constituents are bracketed.

Name.	Source.	Constituents.
Anise oil.	<i>Pimpinella anisum</i> .	Anethole, estragol (anise aldehyde and 'anise ketone').
Bay oil	<i>Pimenta acris</i> .	Eugenol, myrcene $C_{10}H_{16}$ , chavicol, methyl eugenol, estragol, phellandrene.
Bergamot oil.	<i>Citrus bergamea</i> .	Linalyl acetate, linalol, $\delta$ -limonene, bergaptene $C_{12}H_{20}O_4$ .
Cassia oil.	<i>Cinnamomum cassia</i> .	Cinnamic aldehyde, cinnamyl acetate (cumaric aldehyde, methyl ether).
Caraway oil.	<i>Carum carvi</i> .	Carvone ( $\delta$ -limonene).
Camphor oil.	<i>Cinnamomum camphora</i> .	$\delta$ -Pinene, phellandrene, dipentepe, cadinene $C_{15}H_{24}$ , eugenol, safrole, terpineol, acetaldehyde, cineol.
Chamomile oil (Roman).	<i>Anthem. nobilis</i> .	Isobutyl, isoamyl, and hexyl esters of isobutyric, angelic, and tiglic acids.
Cinnamon oil (Ceylon).	<i>Cinnamomum Zeylanicum</i> .	Cinnamic aldehyde (eugenol).
Clove oil.	<i>Eugenia caryophyllata</i> .	Eugenol (caryophyllene $C_{15}H_{24}$ , eugenol acetate, furfural, methyl alcohol, salicylic acid).
Coriander oil.	<i>Coriandrum sativum</i> .	Linalol ( $\delta$ -pinene).
Cumin oil.	<i>Cuminum cymium</i> .	Cumic aldehyde (cuminol), $\gamma$ -mene.
Eucalyptus oil.	<i>Eucalyptus globulus</i> .	Cineol, $\delta$ -pinene (butyric, valeric, and caproic aldehydes).
Fennel oil.	<i>Foeniculum vulgare</i> .	Anethole, fenchone, dipentepe, $\delta$ -pinene.
Geranium oil (East Indian).	<i>Andropogon schoenanthus</i> .	Geraniol, citronello.
Geranium rose oil.	<i>Pelargonium odorat.</i>	Geraniol, citronello.
Jasmine oil.	<i>Jasminum grandiflorum</i> .	Benzyl acetate, linalol, benzyl alcohol, linalyl acetate, methyl anthranilate, indole.

Name.	Source.	Constituents
Lavender oil.	Lavandula vera.	<i>l</i> -Linalyl acetate, linalol (pinene, cineol).
Lemon oil.	Citrus limonum.	Limonene, phellandrene, citral, citronellal, geranyl acetate, linalol.
Lemon-grass oil.	Andropogon citratus.	Citral (citronellal, methylheptenone, geraniol).
Neroli oil.	Citrus bigardia (blossoms).	<i>l</i> -Linalol, linalyl acetate, geraniol, methyl anthranilate, limonene.
Orange oil.	Citrus aurantium (rind).	<i>d</i> -Limonene (citral, citronellal, methyl anthranilate).
Peppermint oil.	Mentha piperita.	Menthol and menthyl esters of acetic, valeric, and other acids, menthone (pinene, phellandrene, <i>l</i> -limonene, cineol, cadinene $C_{15}H_{24}$ , acetic and isovaleric aldehyde, methyl sulphide and ammyl alcohol).
Pine-needle oil.	Pinus sylvestris.	<i>d</i> -Pinene, <i>d</i> -sylvestrene.
Rose oil.	Rosa damascena.	Geraniol, <i>l</i> -citronellal (geranyl acetate).
Rosemary oil.	Rosamarinus officinalis.	Pinene, camphene, cineol, camphor, borneol.
Sage oil.	Salvia officinalis.	Pinene, cineol, thujone, borneol.
Sassafras oil.	Sassafras officinalis.	Safrole (pinene, phellandrene, camphor, eugenol).
Spearmint oil.	Mentha viridia.	<i>l</i> -Linalol, <i>l</i> -carvone (cineol, <i>l</i> -limonene).
Star anise oil.	Illicium anisatum.	Anethole ( <i>d</i> -pinene, <i>l</i> -phellandrene, estragol, quinol, ethyl ether, safrole).
Tansy oil.	Tanacetum vulgare.	Thujone (camphor, borneol).
Thyme oil.	Thymus vulgaris.	Thymol or carvacrol (cymene, <i>l</i> -pinene, borneol, linalol).
Wormwood oil.	Artemisia absinthum.	Thujone, thujyl alcohol, free and combined with acetic, isovaleric, and palmitic acids, phellandrene and cadinene.
Ylang-ylang oil.	Cananga odorata (flowers).	<i>l</i> -Linalol, geraniol, benzoic ester, <i>p</i> -cresol, methyl ether, cadinene.

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## CHAPTER VI

### THE ALKALOIDS

AMONG vegetable products numerous oily and crystalline basic substances termed *alkaloids* have been found, which, in consequence of very marked physiological properties, have been objects of special interest to the chemist and physiologist. The first of these substances to be isolated was a crystalline compound obtained from opium by Derosne in 1803, and called by him *opium salt*, but he failed to recognize its basic character. In 1806 Sertürner, a German apothecary of Eimbeck, independently discovered the same crystalline substance, which he called *morphium* and pointed out its alkaline nature. At the same time he separated an acid which he called *meconic acid*, and expressed the view that the two substances existed in opium in combination. This investigation remained unnoticed at the time; but a second paper published by him in 1817, 'Ueber das Morphin, eine neue salzfähige Grundlage und die Mekonsäure als Hauptbestandtheile des Opiums,' attracted the attention of chemists, who thereupon began to search among vegetable products for similar substances. Success attended their efforts. In the same year Robiquet found narcotine in opium; in 1818 Pelletier and Caventou obtained strychnine from *nux vomica*; in the following year they separated brucine, and in 1820 they prepared quinine and cinchonine from cinchona bark. Since then scarcely a year has passed without the discovery of one or more alkaloids. At the present time the number exceeds two hundred, and the field is not exhausted.

These vegetable bases, all of which contain nitrogen, were regarded as conjugated ammonias by Berzelius (Part I, p. 33), and as substituted ammonias by Liebig and Hofmann, the latter recognizing the majority of them as tertiary bases. Numerous attempts to carry the investigation of these compounds further, and to explain their structure, proved unsuccessful, until it was discovered that certain basic oils, found by Anderson in bone-oil and by Runge and Greville Williams in coal-tar, were identical with the compounds obtained by Gerhardt by distilling some of the alkaloids with caustic potash. The sequence of events was as follows: In 1834 Runge separated the

substance which he termed *leucol* from coal-tar; in 1846 Anderson isolated pyridine and its homologues from bone-oil. Meanwhile Gerhardt (1842) had been subjecting strychnine, cinchonine, and quinine to distillation with solid caustic potash, and obtained an oil which he called quinoleine, afterwards altered to quinoline. Hofmann soon recognized in Runge's leucol and Gerhardt's quinoline identical substances. Subsequently other alkaloids—nicotine, conine, piperine, &c.—were converted into pyridine or one of its derivatives by heating with zinc dust. Isoquinoline, which was discovered in coal-tar in 1885 by Hoogewerff and van Dorp, has been shown to be related in a similar way to hydrastine, papaverine, narcotine, and berberine.

These discoveries, whilst they gave a fresh stimulus to the investigation of the alkaloids, opened up a new field for research in the study of pyridine and quinoline derivatives. The result has been that the process of graduated disintegration applied to the alkaloids on the one hand, and the construction of new products from pyridine and quinoline on the other, established points of contact between them which gradually disclosed the structure of many of the alkaloids, and ultimately led to the synthesis of a few of them.

The history of these successive stages is the main object of the present chapter. In the light of this new knowledge, how is the term alkaloid to be defined? Koenigs suggested that the name, which was originally applied to all vegetable bases, including caffeine, theobromine, betaine, choline, &c., should be restricted to those vegetable products which are derivatives of pyridine only. This would exclude caffeine and theobromine, which do not differ very widely from the alkaloidal bases. In the present state of the subject an exact definition is not easy to frame, and possibly, as our knowledge grows, the line of demarcation between the alkaloids at present known and other vegetable products may become gradually obliterated. For the present, however, an alkaloid may be defined as a vegetable base which contains a cyclic nitrogenous nucleus.

Before passing to the more complex alkaloids, a brief review of the parent substances seems desirable, and we propose, therefore, to describe briefly the properties and structure of pyridine, quinoline, and isoquinoline, and their more important derivatives, before passing to the chemistry of the alkaloids. It would be beyond the scope of these essays to attempt more than a general description of these substances, the number being already vast enough to have filled a volume of respectable dimensions.<sup>1</sup>

<sup>1</sup> See References at the end of the chapter.

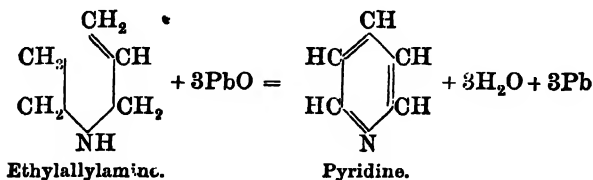
**Pyridine.** It has already been stated that pyridine and its homologues were first obtained by Anderson from bone-oil. This oil, which is formed by the destructive distillation of bones, contains a large number of compounds derived from the decomposition of the albuminoid material of the bone. The bases are extracted from the distillate with acid, liberated by the addition of alkali and finally fractionated. The chief source of pyridine at present is coal-tar-naphtha, from which it is separated by sulphuric acid employed in purifying the naphtha. It is also formed by the action of alkalis or zinc dust at a high temperature on several of the alkaloids—nicotine, morphine, cinchonine, &c.—by the oxidation of piperidine (hexahydropyridine, see p. 291) with strong sulphuric acid at 300° or nitrobenzene at 250°, and by distilling the lime salts of pyridine carboxylic acids with lime. It has, moreover, been obtained synthetically by the following methods, all of which, it may be added, give unsatisfactory yields. This is not the case with the reactions to be described later for the preparation of certain pyridine derivatives.

In 1877 Ramsay,<sup>1</sup> following the lines of Berthelot's synthesis of benzene from acetylene, passed a mixture of acetylene and hydrocyanic acid through a red-hot tube and obtained small quantities of pyridine.



Koenigs<sup>2</sup> in 1879 was successful in obtaining some pyridine by distilling ethylallylamine over hot litharge.

Using the generally accepted formula for pyridine (it is discussed later, p. 268), the following represents the reaction :



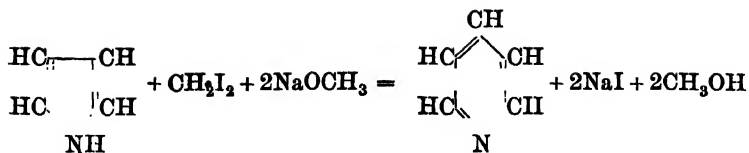
In 1881 Ciamician and Dennstedt<sup>3</sup> acted upon pyrrole with sodium ethylate and chloroform or bromoform and obtained a  $\beta$ -chloro- and  $\beta$ -bromo-pyridine. In 1885 Dennstedt and Zimmermann using methylene iodide obtained pyridine.<sup>4</sup> The action is a curious one, and can only be formulated by supposing the pyrrole ring to open and absorb an additional carbon group.

<sup>1</sup> Ber., 1877, 10, 736.

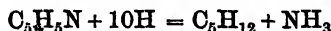
<sup>2</sup> Ber., 1881, 14, 1153.

<sup>3</sup> Ber., 1879, 12, 2344.

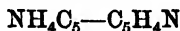
<sup>4</sup> Gr., 1885, 18, 3316.



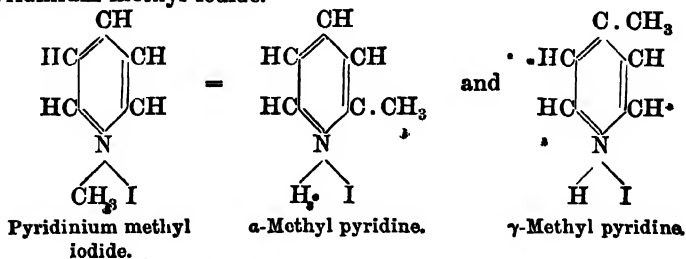
Pyridine is a colourless liquid, possessing a peculiar smell. It boils at  $114.8^\circ$ , and has nearly the same specific gravity as water. It is easily soluble in water as well as in most of the common solvents, and forms crystalline salts and double salts like other organic bases. It is peculiarly stable towards oxidising agents, chromic and strong or weak nitric acid having little or no action. The halogens also react with difficulty, so that the halogen derivatives, as well as amino compounds, are usually prepared by indirect methods, which will be described later. Strong or fuming sulphuric acid has little action at ordinary temperatures, but a sulphonic acid can be obtained by heating pyridine with the fuming acid at  $300^\circ$ . By the action of sodium on an alcoholic solution of pyridine, hexahydropyridine (piperidine) is obtained, whereas hydriodic acid at a high temperature decomposes it into normal pentane and ammonia.



Sodium alone removes an atom of hydrogen from pyridine, and dihydridyl is formed by the linking of two molecules,



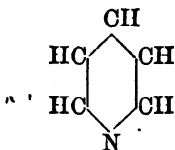
Pyridine is a tertiary base, and therefore forms quaternary compounds with the alkyl halides. These bodies undergo molecular change on heating to  $300^\circ$ , resembling the decomposition of the hydrochlorides of the monoalkyl anilines. Just as *o*- and *p*-toluidine are produced by heating methylaniline hydrochloride under pressure (Part II, p. 369), so  $\alpha$ - and  $\gamma$ -methyl pyridine are obtained from pyridinium methyl iodide.



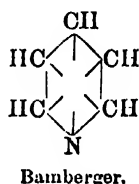
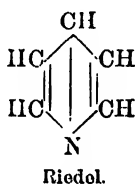
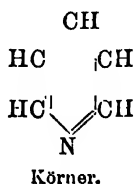
This reaction has furnished a number of important homologues of pyridine.



The great stability of pyridine, its analogy in chemical behaviour with benzene, the formation of a hexahydride, and the existence of three mono-substitution derivatives point to the structure of pyridine as a ring of six atoms, of which five are carbon and one is nitrogen. Each of the carbon atoms being united to one atom of hydrogen,

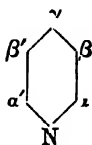


there remain six single valencies which have been accounted for by the use of different formulae. Körner in 1869 first suggested the application of Kekulé's formula to pyridine; this was followed by the formula of Riedel in 1883 and of Bamberger in 1891.



There are arguments which may be adduced in favour of all three formulae; but the experimental data are at present too incomplete to afford a final decision. As the constitution of pyridine is closely interwoven with that of quinoline, we have deferred its discussion to the end of the present section (p. 281). In the meantime we shall adopt the Körner formula.

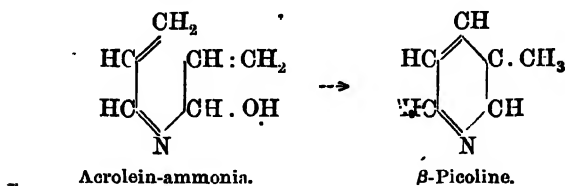
The substitution products of pyridine are denoted by the Greek letters  $\alpha$ ,  $\beta$ ,  $\gamma$  or by numbers.



All three methyl and ethyl pyridines, hydroxy-, chloro-, and amino-pyridines and pyridine carboxylic acids are known.

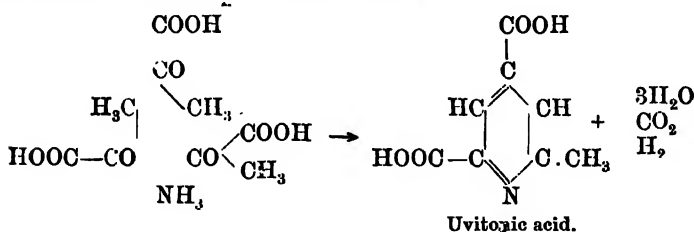
The alkyl pyridines resemble pyridine in most of their chemical properties, and, like the homologues of benzene, the side-chains are oxidisable, and acids result. The origin of some of these compounds has already been mentioned. All three methyl pyridines or picolines

are present in bone-oil. Baeyer<sup>1</sup> found that the resin obtained by the action of ammonia upon acrolein yields on distillation  $\beta$ -picoline. The constitution of acrolein-ammonia is not definitely known, but the formula and its transformation into picoline may probably be represented as follows:

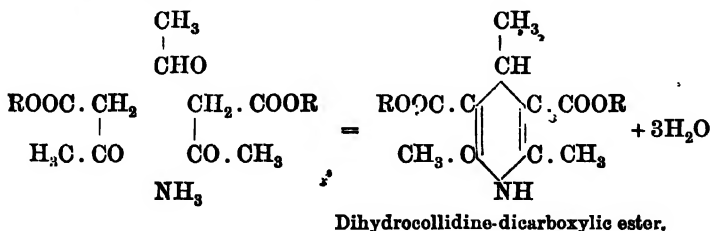


This method was the forerunner of others in which aldehyde-ammonias with or without the addition of an aldehyde or ketone were employed. We select two examples, the formation of  $\beta$ -picoline by Böttinger<sup>2</sup> and that of collidine (*s*-trimethylpyridine) by Hantzsch.<sup>3</sup>

By the action of ammonia on pyruvic acid a dibasic acid, uvitonic acid  $\text{C}_6\text{H}_5\text{N}(\text{COOH})_2$ , is formed, which, on distillation with lime, loses carbon dioxide and yields  $\alpha$ -picoline.



By heating together gently for a few minutes two equivalents of acetoacetic ester with one of acetaldehyde-ammonia, dihydrocollidine-dicarboxylic ester results.



<sup>1</sup> *Annalen*, 1870, 155, 281.

<sup>2</sup> *Ber.*, 1877,<sup>4</sup> 10, 362; 1890, 13, 2032.

<sup>3</sup> *Ber.*, 1882, 15, 2914.

This compound, on oxidation with nitrous acid, loses two atoms of hydrogen, forming collidine-carboxylic ester, from which, on hydrolysis and distillation with lime, collidine is obtained.

Special interest attaches to those alkyl pyridines which are derived from the alkaloids themselves. By distilling conine hydrochloride with zinc dust, Hofmann obtained a base boiling at 166–168°, which he termed *conyrine*, and which has since been identified as  $\alpha$ -propyl pyridine.

Nicotine passed through a red-hot tube yields  $\beta$ -ethyl pyridine and  $\beta$ -propyl pyridine, whereas the hydrochloride of norhydrotropidine, a decomposition product of atropine, gives on distillation with zinc dust  $\alpha$ -ethyl pyridine. Williams obtained  $\beta$ -ethyl pyridine by distilling cinchonine and quinine with caustic potash. Instances of the same thing might be multiplied.

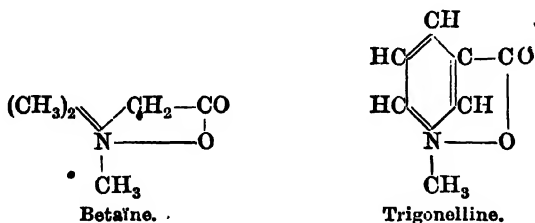
The pyridine carboxylic acids have also played an important part in fixing the structure of the alkaloids. As the degradation of the alkaloids by oxidation generally results in the production of one or other of these acids, it has been a matter of the first importance to determine the position of the carboxyl groups in the pyridine nucleus. This has been satisfactorily accomplished by methods for a description of which the reader is referred to one of the larger textbooks. The methods of preparation are the same as those used in the case of the acids of benzene, namely, the oxidation of side-chains or the hydrolysis of the nitriles.

The following acids have a special interest, arising from their direct or indirect connection with the alkaloids: *Nicotinic acid* ( $\beta$ -pyridine carboxylic acid) is obtained from nicotine, pilocarpine, hydrastine, and berberine by oxidation and by the action of hydrochloric acid on trigonelline (see below); the following are also obtained by oxidation: *quinolinic acid* ( $\alpha$ - $\beta$ -pyridine dicarboxylic acid) from quinoline; *cinchomeronic acid* ( $\beta$ - $\gamma$ -pyridine dicarboxylic acid) from isoquinoline,  *$\alpha$ -carbocinchomeronic acid* ( $\alpha$ - $\beta$ - $\gamma$ -pyridine tricarboxylic acid) from several of the cinchona alkaloids, and papaverine and berberonic acid ( $\beta$ - $\gamma$ - $\alpha'$ -pyridine tricarboxylic acid) from berberine.

The  $\alpha$ -carboxylic acids occupy a distinctive position among the pyridine acids, owing to the facility with which they lose carbon dioxide on heating, and also by reason of the yellow colour reaction which they give with ferrous sulphate. Although the pyridine acids naturally possess basic as well as acid characters, the usual reactions of the carboxyl group are not thereby affected. From the acids the corresponding amide and, by means of Hofmann's hypobromite

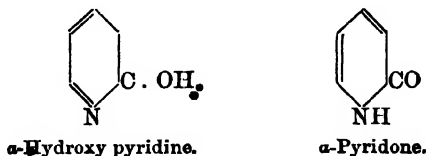
reaction, the amino pyridines may be prepared. The latter resemble the aliphatic amines rather than the aromatic amino compounds; for only the  $\beta$ -compound can be diazotised with nitrous acid, and manifests the usual reactions of diazo compounds.

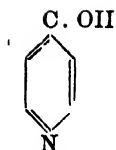
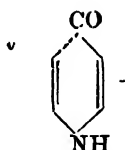
It has been stated that trigonelline is converted into nicotinic acid by the action of hydrochloric acid. This alkaloid, which is found in fenugreek (*trigonella foenum-graecum*), represents a new and interesting class of compound now recognized by the generic term of *betaines*. Betaine has long been known as a constituent of beetroot sap, and its constitution has been fixed by synthesis as the inner anhydride of hydroxytrimethylglycocoll. In 1885, Jahns<sup>1</sup> discovered trigonelline in the seeds of fenugreek, and showed that the substance was identical with a compound obtained by Hantzsch<sup>2</sup> from nicotinic acid, by treating the acid successively with methyl iodide and silver hydroxide. The relationship between these two substances is readily understood from the following formulae:



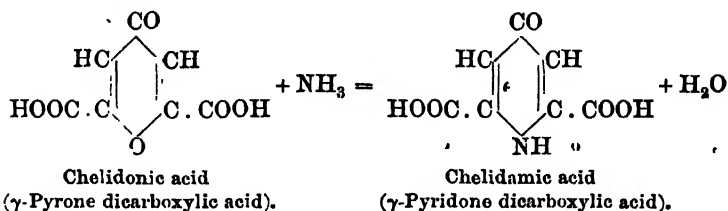
Similar compounds have been found among the oxidation products of cotarnine and hydrastinine.

The only other group of compounds to which reference need be made are the hydroxy pyridines. These substances possess both basic and phenolic properties and correspond to the amino phenols. The  $\alpha$ - and  $\gamma$ -compound, but not the  $\beta$ -compounds, are also characterized by their behaviour as ketones or lactams, in other words, they exhibit the property of tautomerism, and may, therefore be represented by the following double formulae:

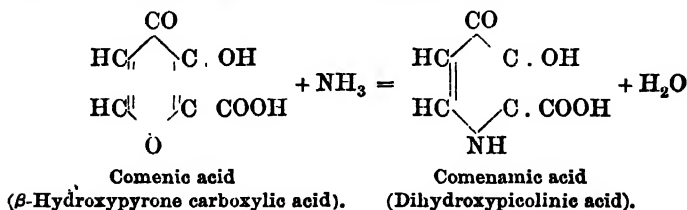


 $\gamma$ -Hydroxy pyridine. $\gamma$ -Pyridone.

They are usually obtained by removing carboxyl from the hydroxypyridine carboxylic acids by distillation with lime. The  $\alpha$ - and  $\gamma$ -compounds may also be prepared from the derivatives of  $\alpha$ - and  $\gamma$ -pyrones by the action of ammonia in the cold, the single oxygen atom being replaced by the NH group. This connection between the pyrones and hydroxypyridines is a peculiarly interesting one, since pyrone compounds, associated with alkaloids, are widely distributed among plants. Thus, Lieben and Haitinger<sup>1</sup> found that chelidonic acid, which occurs with the alkaloid chelidonine in the root of common celandine, is converted into chelidamic or  $\gamma$ -pyridone dicarboxylic acid.



Ost<sup>2</sup> obtained in the same way from comenic acid (hydroxy-pyrone carboxylic acid), which is formed by heating meconic acid of opium, comenamic acid, or dihydroxypicolinic acid.



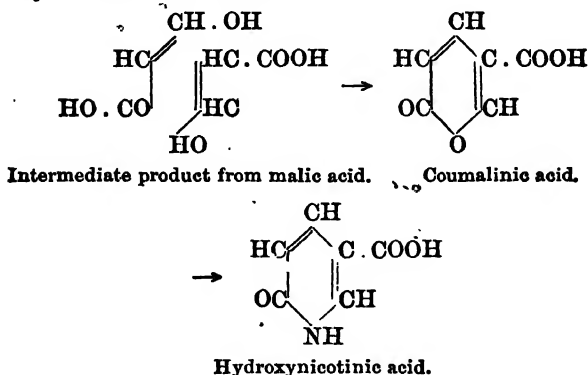
Another interesting synthesis of the same character is the formation of hydroxynicotinic acid from coumalinic acid.<sup>3</sup> Coumalinic acid is obtained by the action of strong sulphuric acid on malic acid,

<sup>1</sup> *Monatsh.*, 1883, 4, 275; 1885, 6, 279.

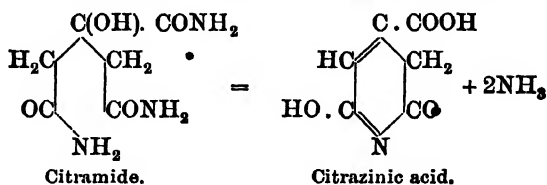
<sup>2</sup> *J. prakt. Chem.*, 1883, 21, 257; 1884, 29, 57, 378.

<sup>3</sup> Von Pechmann and Welch, *Ber.*, 1884, 17, 936, 2384; 1885, 18, 817.

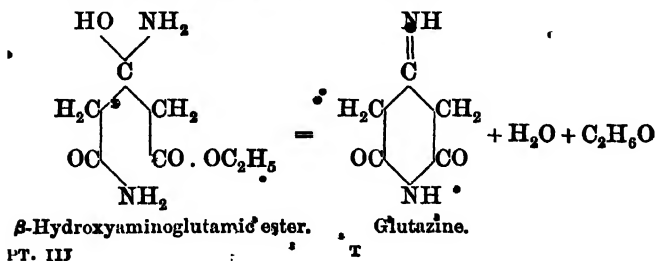
in which the intermediate formation of hydroxymethylene acetic acid may be assumed to occur.



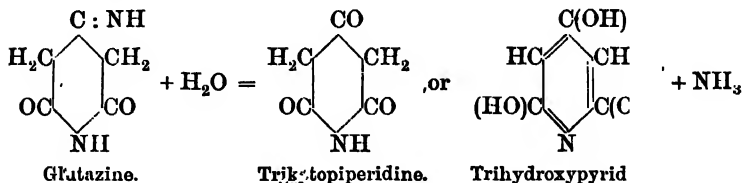
If, as seems on the whole not improbable, the pyrones form the basis of some of the alkaloids, a simple mode of preparing pyrone compounds may throw some light on the initial stages of the synthetic process by which these complex molecules are elaborated in the plant. Di- and tri-hydroxypyridines and hydroxypyridine carboxylic acids have also been obtained indirectly from citric acid. Thus, hydrochloric acid converts citramide or one of the amides of citric acid into citrazinic acid (*aa'*-dihydroxynicotinic acid).



Glutazine (*aa'*-dihydroxy- $\gamma$ -amino pyridine) is obtained by the acting on acetonedicarboxylic ester with ammonia and boiling the product,  $\beta$ -hydroxyaminoglutamic ester, with soda solution.



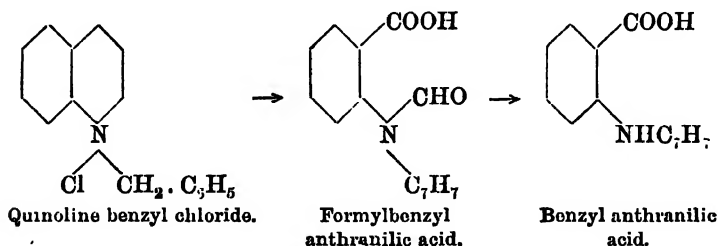
By boiling glutazine with hydrochloric acid, triketopiperidine or  $\alpha\alpha'\gamma$ -trihydroxypyridine is produced.



As acetonedicarboxylic ester is a product of the action of sulphuric acid on citric acid, both this and the previous product may be regarded as indirectly obtained from citric acid.

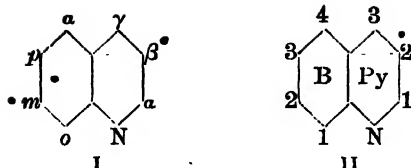
**Quinoline.** In chemical properties quinoline offers many points of resemblance to pyridine. It is a colourless, oily liquid, specifically heavier than water, in which, unlike pyridine, it is only slightly soluble. It boils at  $240^\circ$ . On reduction it takes up 4, 8, and finally 10 additional atoms of hydrogen, forming decahydroquinoline.

Quinoline bears very much the same relation to pyridine that naphthalene does to benzene. Thus, on oxidation with permanganate solution, quinolinic acid, i. e.  $\alpha\beta$ -pyridine dicarboxylic acid, is formed, whereas the additive product of quinoline with benzyl chloride gives under similar conditions a mixture of the benzyl derivatives of anthranilic acid and formyl anthranilic acid.



It seems as if the ring containing nitrogen in its quinquevalent state were rendered less resistant to oxidising agents.

The appearance of a pyridine nucleus in the product of the first process and of a benzene nucleus in that of the second has led to the hypothesis of a double hexagon formula for quinoline, consisting of a benzene and pyridine ring having two carbon atoms common to both.

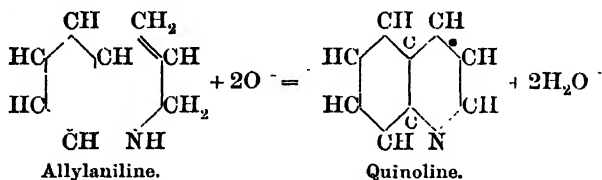


This arrangement of the atoms would also agree perfectly well with the various syntheses of quinoline and its derivatives described below; but a fuller discussion of the subject is reserved for the conclusion of the section (p. 281).

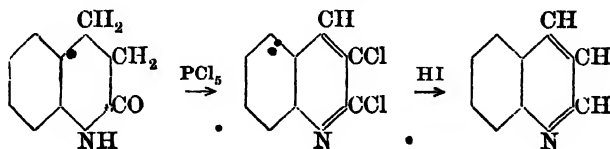
The seven hydrogen atoms, according to the hexagon formula, are evenly distributed among the carbon atoms, which are distinguished by the Greek letters  $\alpha$ ,  $\beta$ ,  $\gamma$  in the pyridine nucleus, and by ortho, meta, para, and ana in the benzene ring (I), or, according to another method, by numbers following the initial letters B or Py, to indicate the benzene or pyridine nucleus respectively (II). Seven mono derivatives of quinoline are therefore possible, and of these all the methyl quinolines and quinoline carboxylic acids, and six of the seven hydroxyquinolines, are known.

Quinoline and some of its homologues are obtained from coal-tar, from certain alkaloids by distillation with potash, or by means of one or other of numerous synthetic methods from which the following are selected:

In 1879 Koenigs<sup>1</sup> obtained quinoline, in a similar manner to that by which he prepared pyridine, by passing the vapour of allylaniline over heated lead oxide.



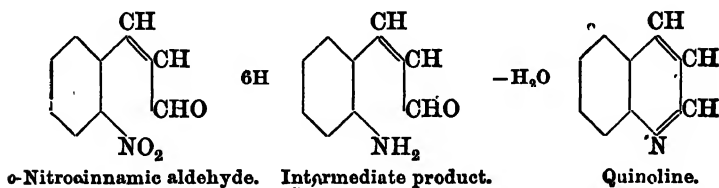
In the same year Baeyer converted hydrocarbostyryl (the anhydride of *o*-aminohydrocinnamic acid) into quinoline by the successive action of phosphorus pentachloride and hydriodic acid.



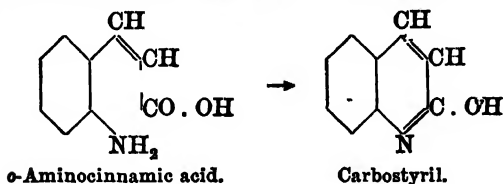
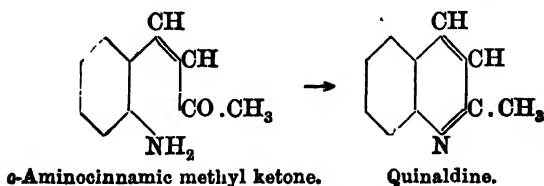
<sup>1</sup> Ber., 1879, 12, 453.



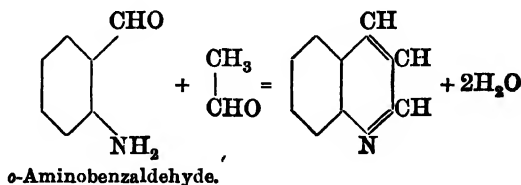
This method was afterwards modified,<sup>1</sup> and in place of aminocinnamic acid, *o*-nitrocinnamic aldehyde was reduced.



To the same category of reactions belong the formation of quinaldine ( $\alpha$ -methyl quinoline) from *o*-aminocinnamic ketone and carbostyryl ( $\alpha$ -hydroxyquinoline) from *o*-aminocinnamic acid.



A further interesting development of the method is due to Friedländer, who in 1883<sup>2</sup> obtained quinoline by condensing *o*-aminobenzaldehyde with acetaldehyde in presence of a little caustic soda solution.

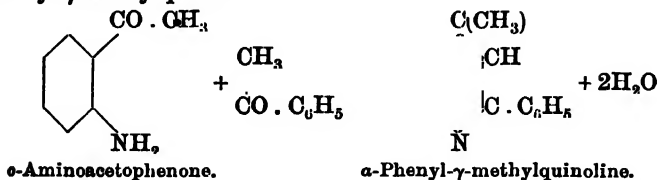


In place of acetaldehyde, a variety of aldehydes and ketones may be used, and even the aldehyde group of the basic constituent may be replaced by a ketone.

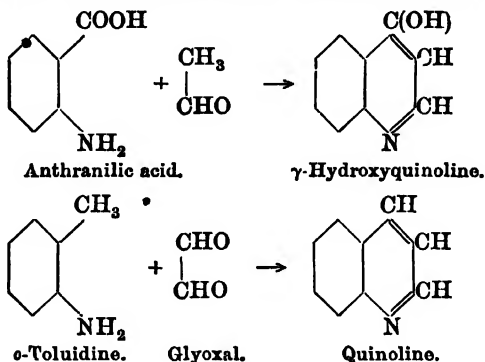
<sup>1</sup> Ber., 1883, 16, 2207.

<sup>2</sup> Ber., 1882, 15, 2572; 1883, 16, 1833.

To take one example, *o*-aminoacetophenone gives with acetophenone,  $\alpha$ -phenyl- $\gamma$ -methylquinoline.

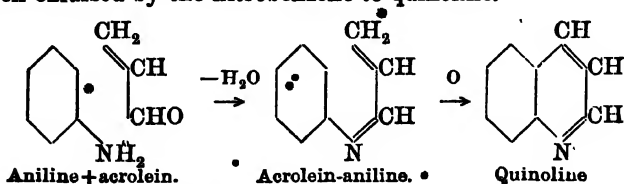


Anthranilic acid and *o*-toluidine have also been used in conjunction with aldehydes for effecting quinoline synthesis.



To a somewhat different class of reactions belongs the important synthetic method of Skraup.

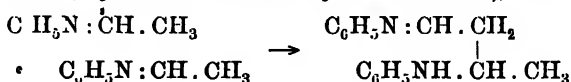
In 1880 Koenigs obtained quinoline by heating nitrobenzene with glycerol and sulphuric acid at 180–190°, a reaction which was very soon replaced by the more effective process of Skraup,<sup>1</sup> which appeared in the same year. It consists in heating an aromatic amino compound with glycerol, sulphuric acid, and nitrobenzene. The mechanism of Skraup's method is usually explained by assuming that glycerol undergoes conversion into acrolein by the dehydrating action of the acid, and is followed by the formation of acrolein-aniline with the aniline or aniline derivative. This acrolein-aniline is then oxidised by the nitrobenzene to quinoline.



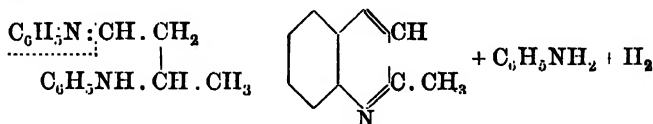
<sup>1</sup> *Monatsh.*, 1880, 1, 316; 1881, 2, 141.

The method is of the greatest value, for, provided one ortho position to the amino group in the nucleus is free, any amino compound may be used, and the number of substituted quinolines which can be prepared in this way is very large. The substituent group in these cases is naturally restricted to the benzene nucleus.

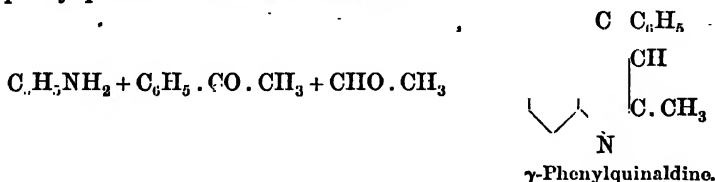
The quinaldine synthesis of Doebner and von Miller must complete this brief summary of synthetic methods. It consists in the action of sulphuric or hydrochloric acid upon a mixture of aniline and aldehydes. From aniline and acetaldehyde (or paraldehyde)  $\alpha$ -methylquinoline or quinaldine is obtained. The mechanism of the process probably consists in the condensation of two molecules of alkylidene aniline (formed by union of the aldehyde and aniline), thus:



It is followed by the elimination of aniline and hydrogen with ring formation.



In place of aldehyde or ketone, mixtures of aldehydes and ketones may be used. With aniline, acetophenone, and acetaldehyde,  $\gamma$ -phenylquinaldine has been obtained.



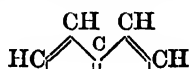
An adequate account of the chemistry of quinoline and its derivatives would carry us far beyond the scope of the present essay, which is intended to include only those compounds possessing some kind of relation to the alkaloids.

Numerous alkyl quinolines have been prepared synthetically by one of the methods already described and possess the general characteristics of quinoline. Lepidine or  $\gamma$ -methylquinoline can be obtained from cinchonine by distillation with potassium hydroxide or lead oxide, and it is also interesting to note that *p*-methoxyepidine occurs among the decomposition products of quinine.  $\gamma$ -Phenylquinoline is another product which brings us into touch with the

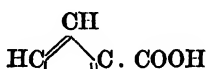
alkaloids, for, according to Koenigs, it is the parent substance of quinine.

The following quinoline acids are also directly related to the alkaloids: *cinchoninic acid* ( $\gamma$ -quinoline monocarboxylic acid) is obtained by oxidising several of the cinchona alkaloids, and *quininic acid* (*p*-methoxycinchoninic acid) is formed by the oxidation of quinine.

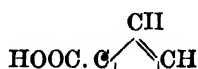
**Isoquinoline**, which is isomeric with quinoline, is a colourless solid, melting at  $21^\circ$ , boiling at  $240^\circ$ , and possessing a smell like quinoline. It was originally found by Hoogewerff and van Dorp<sup>1</sup> in coal-tar in the crude quinoline fraction and separated by crystallization of the slightly soluble sulphate. On oxidation it gives both phthalic and cinchomeronic acid, which points to the following structural formula:



Isoquinoline.

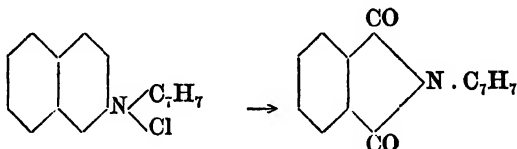


Phthalic acid.

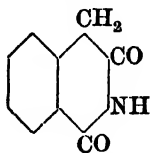


Cinchomeronic acid.

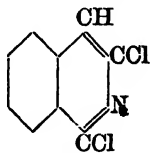
Also alkyl isoquinolinium halides give on oxidation alkyl phthalimides.



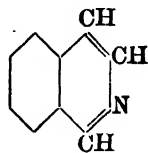
This structure has been confirmed by subsequent synthesis, which was first accomplished in 1886 by Gabriel. Homophthalimide is converted by phosphorus pentachloride into dichloroisoquinoline, which on further heating with hydriodic acid and phosphorus passes into isoquinoline, identical with Hoogewerff and van Dorp's base.



Homophthalimide.



Dichloroisoquinoline.



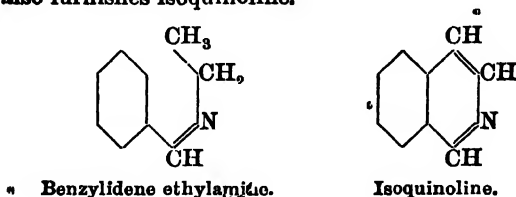
Isoquinoline.

Le Blanc<sup>2</sup> has effected the same result more directly by heating homophthalimide with zinc dust in a current of hydrogen. A

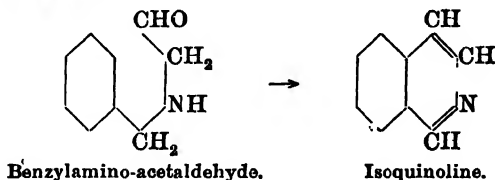
<sup>1</sup> *Rec. trav. chim.*, 1885, 4, 125.

<sup>2</sup> *Ber.*, 1888, 21, 2299.

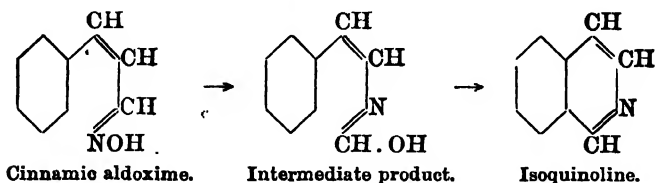
reaction, resembling the formation of quinoline from allylaniline, which consists in passing benzylidene ethylamine through a red-hot tube, also furnishes isoquinoline.



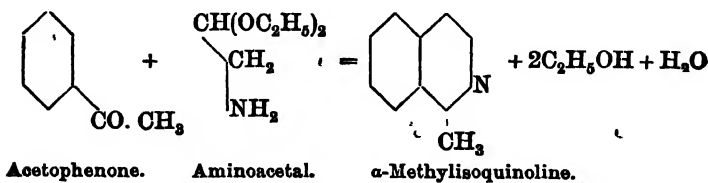
Fischer<sup>1</sup> obtained isoquinoline by dissolving benzylamino-acetaldehyde in fuming sulphuric acid, which acts both as a dehydrating and oxidising agent.



An interesting isoquinoline synthesis was effected by Bamberger and Goldschmidt,<sup>2</sup> who found that both stereoisomers of cinnamic-aldoxime on distillation with phosphorus pentoxide undergo the Beckmann change and give isoquinoline as follows (Part II, p. 366):



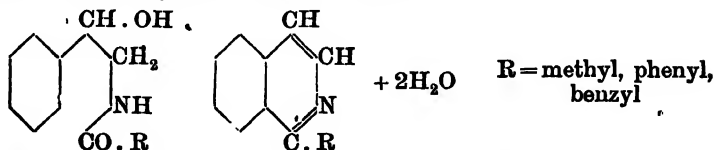
Pomeranz obtained  $\alpha$ -methylisoquinoline by the action of strong sulphuric acid on a mixture of aminoacetal and acetophenone.



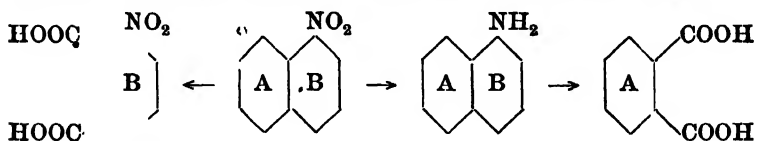
<sup>1</sup> Ber., 1893, 26, 764.

<sup>2</sup> Ber., 1894, 27, 1954, 2795.

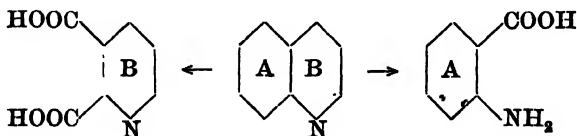
Isoquinoline derivatives may also be prepared by condensing acyl carbinols with phosphoric acid in xylene.<sup>1</sup>



**Structure of Pyridine, Quinoline, and Isoquinoline.** In discussing in further detail the constitution of pyridine, quinoline, and isoquinoline, that of naphthalene may be included, since the same process of reasoning has been applied in turn to each. Assuming that benzene and pyridine form rings of six atoms, it follows that quinoline, isoquinoline, and naphthalene are each composed of a double nucleus of six atoms, two of which are common to both nuclei. For, in the case of naphthalene, it has been shown that if either nucleus is removed by oxidation a benzene derivative results. Graebe found that if  $\alpha$ -nitronaphthalene is oxidised, nitrophthalic acid is formed, whereas  $\alpha$ -naphthylamine, obtained from the same  $\alpha$ -nitronaphthalene by reduction, yields phthalic acid.



In the one case nucleus A is removed, in the other nucleus B, and in both cases a benzene nucleus remains. Quinoline in the same way may be oxidised to quinolinic acid on the one hand, and to a derivative of anthranilic acid on the other (p. 274).



Under similar conditions isoquinoline gives a mixture of phthalic and cinchomeronic acid (p. 279).

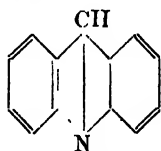


<sup>1</sup> Picot and Gams, 1910, 43, 2384.

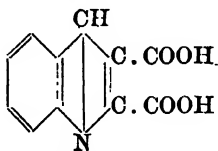
The symmetrical grouping of the hydrogen atoms is borne out by the existence of two isomeric mono derivatives of naphthalene, three of pyridine and seven of quinoline, whereas the equivalence of the two nuclei in the case of naphthalene follows from the fact that 2.7-dihydroxynaphthalene forms with alcohol and sulphuric acid a dialkyl ether. For this property, of forming ethers after the manner of alcohols is not shared by the phenols of benzene, and, as first pointed out by Bamberger, the formation of such an ether in both nuclei of naphthalene is strong evidence in favour of their symmetry.

Having set forth the facts upon which a symmetrical bi-cyclic nucleus is based, the issue narrows itself down to a discussion of the fate of the fourth carbon and third nitrogen bond. As in the case of benzene we have a choice of formulae, firstly, those modelled on Kekulé's formula for benzene, which include Erlenmeyer's naphthalene formula and Körner's formula for pyridine and quinoline, secondly, those modelled on the Armstrong and Baeyer centric formula, which is represented by Bamberger's formula for naphthalene, quinoline, and isoquinoline, and finally Riedel's diagonal formula for pyridine and quinoline.

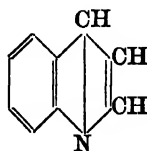
Riedel's formula<sup>1</sup> may be dismissed in a few words. It rests mainly upon the formation of acridinic acid (quinoline dicarboxylic acid) from acridine by oxidation, and certain synthetic methods involving the use of aliphatic compounds. As acridine, like anthracene, is supposed to possess a para linkage connecting the nitrogen and carbon of the middle nucleus, the same kind of linkage is retained in the quinoline and also in the pyridine formula.



Acridine.



Acridinic acid.



Quinoline.

Such an assumption, quite apart from the uncertainty which surrounds the structure of acridine itself, implies an immobility of the para linkage during the degradation of the molecule which does not necessarily follow. The formula is moreover difficult to reconcile with the mechanism of those synthetic methods by which the majority of pyridine and quinoline derivatives are prepared.

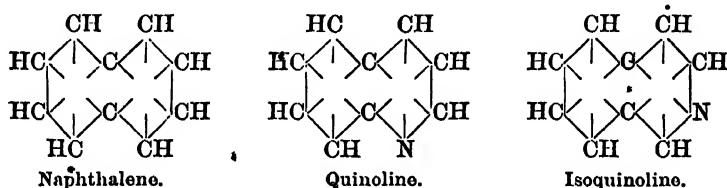
None of these difficulties are presented by either the Kekulé or centric type of formula. At the same time it must be admitted

<sup>1</sup> Ber., 1883, 10, 1609.

that the Kekulé type of formula is open to precisely the same kind of criticism which benzene affords, and which has been fully discussed in Part II, p. 377.

But suppose we accept Kekulé's formula for benzene and pyridine, does the formation of benzene derivatives from naphthalene, and both benzene and pyridine derivatives from quinoline and isoquinoline, necessarily imply the pre-existence of these nuclei in the original compound?

Although at first sight an affirmative reply to the question appears the most simple and obvious one, Bamberger<sup>1</sup> has shown that there are many experimental facts which are opposed to it, and he prefers to regard all three compounds as represented by a ring of ten atoms, in which the fourth carbon, or third nitrogen, bond, as the case may be, are directed towards the centre of each nucleus thus:

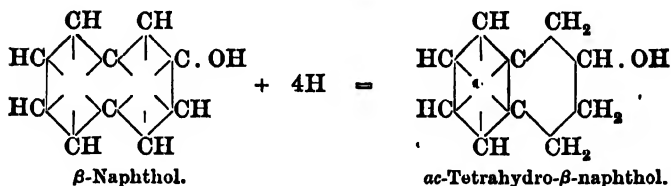
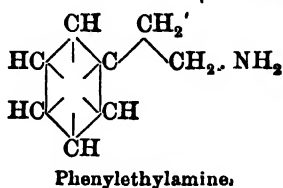
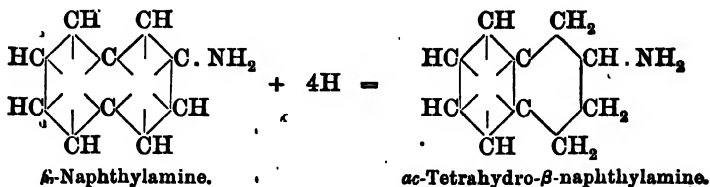


The evidence in favour of this theory will now be briefly reviewed. By the action of sodium in amyl alcohol solution upon  $\alpha$ - and  $\beta$ -naphthol and  $\alpha$ - and  $\beta$ -naphthylamine, Bamberger obtained tetrahydro compounds in which four atoms of hydrogen were added either to the substituted or unsubstituted nucleus. The two hydrogenated compounds, which are formed simultaneously, can be separated by suitable means and their nature determined by the products of oxidation. If the reduced nucleus contains the amino or hydroxyl group, the compound loses its aromatic character and takes that of an aliphatic compound. In this case the compound is termed alicyclic = *ac* ( $\alpha\lambda\epsilon\iota\phi\alpha\rho$ , fat;  $\kappa\acute{\upsilon}\kappa\lambda\omicron\varsigma$ , ring) since it contains a closed chain having aliphatic properties. Thus, *ac*-tetrahydro- $\beta$ -naphthylamine closely resembles phenylethylamine in ammoniacal smell, strongly basic character, and in the stability of its crystalline nitrite; further, in the fact that, by the action of dilute sulphuric acid and hydrobromic acid, ammonia is evolved, and in each case an unsaturated hydrocarbon (styrene in the one case and dihydronaphthalene in the other) results. The corresponding *ac*-tetrahydro- $\beta$ -naphthol has the properties of an alcohol.

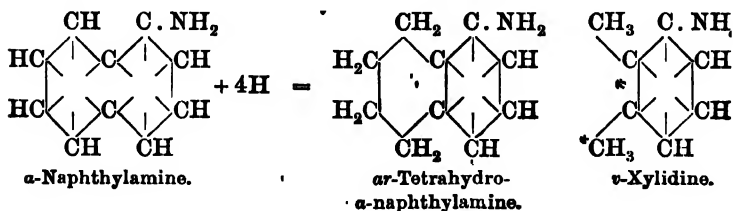
<sup>1</sup> *Annalen*, 1890, 257, 1.



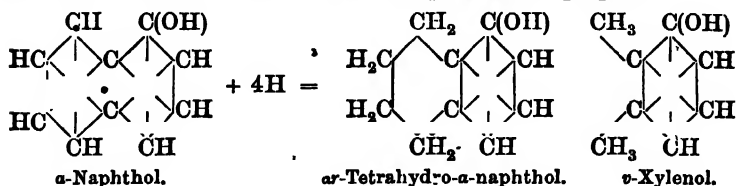
This is accounted for by supposing that in Bamberger's formula the withdrawal of the four free bonds of the substituted nucleus causes the two middle carbon atoms to unite, thereby converting the other nucleus into a benzene ring.



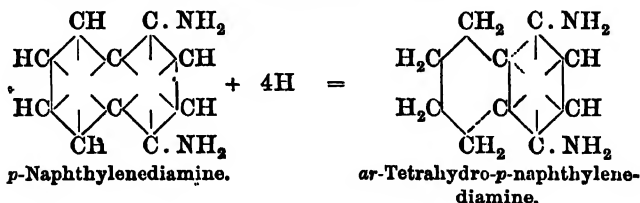
Suppose now that the unsubstituted nucleus has undergone reduction, the benzenoid character of the compound is accentuated, and to distinguish it from the other hydrogenated product it is termed aromatic = *ar*. Thus, *ar*-tetrahydro- $\alpha$ -naphthylamine is much more closely related to *v*-xylidine than to  $\alpha$ -naphthylamine, for unlike the latter it gives no colour reaction with ethyl nitrite. On the other hand, it possesses the neutral reaction, the weak basic properties, and characteristic behaviour towards nitrous acid of an aromatic base. Here the withdrawal of the four bonds transforms the substituted nucleus into a benzene ring.



In the same way xylénol resembles *ar*-tetrahydro-*a*-naphthol, for the latter, like all the phenols derived from benzene, is incapable of forming ethers with alcohol and sulphuric acid, whereas *α*- and *β*-naphthol in common with the alcohols possess this property.



Two more examples must suffice. Para-phenylenediamine can be readily converted into the colouring matters known as indamine, safranin, and thionine dyes. This property is entirely absent in the corresponding naphthalene derivative. If, however, *p*-naphthylenediamine is reduced in the unsubstituted nucleus, the characteristic properties of the benzene derivative immediately appear. This is readily understood from the change of structure.



Again, the naphthoquinones combine with phenylhydrazine to form hydrazones, whilst quinones of the benzene series are merely reduced to the quinols. Hydrogenation in the unsubstituted nucleus produces tetrahydronaphthoquinones which show an exactly parallel behaviour with the benzoquinones and undergo reduction to tetrahydronaphthoquinols.

The results are embodied by Bamberger in the following three propositions:

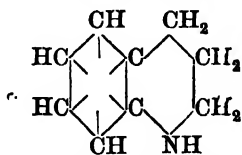
1. In naphthalene and in those derivatives in which each of the eight carbon atoms is linked to a univalent radical (or element) there exist two carbon systems, one of which becomes a benzene ring when the other takes up four atoms of hydrogen.

2. If one of the two carbon systems of naphthalene takes up four atoms of hydrogen, it assumes thereby the functions of an open aliphatic chain.

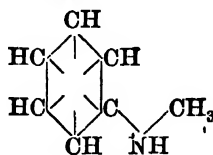
3. The process of hydrogenation of one system consists in converting the product into one resembling a benzene derivative with

an aliphatic side-chain, the hydrogenated part assuming aliphatic, the non-hydrogenated part aromatic functions.

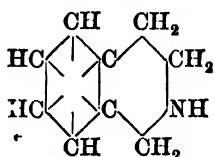
The experience with naphthalene has been found by Bamberger to embrace other cyclic structures. If, for example, the pyridine nucleus in quinoline and isoquinoline is reduced, the tetrahydro compounds so formed have the closest resemblance to methylaniline in the former case and to benzylamine in the latter.



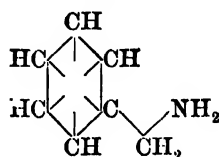
Tetrahydroquinoline.



Methylaniline.

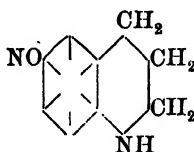
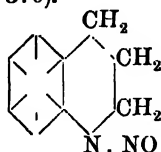


Tetrahydroisoquinoline.

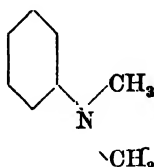
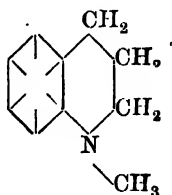


Benzylamine.

The resemblance is exhibited in the following way: Ziegler<sup>1</sup> showed that tetrahydroquinoline nitrosamine undergoes intramolecular change with alcoholic hydrogen chloride in precisely the same way as methylaniline nitrosamine; in both cases the nitroso group is transferred to the para position to the amino group (Part II, p. 370).

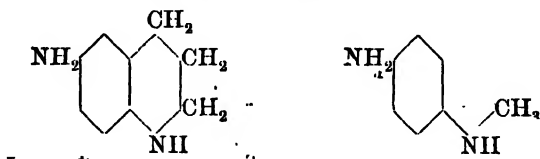


Again, the N-methyl derivative of tetrahydroquinoline behaves like dimethylaniline;



<sup>1</sup> *Ber.*, 1888, 21, 862.

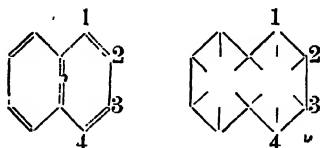
for it yields a *p*-nitroso derivative resembling nitrosodimethylaniline; it forms a corresponding leucomalachite green with benzaldehyde, which like that derived from dimethylaniline yields a green dye-stuff on oxidation, and it unites with diazonium salts to form red azo-colours. Moreover, *p*-aminotetrahydroquinoline gives the characteristic colour changes exhibited by an alkylated *p*-phenylenediamine, such as the



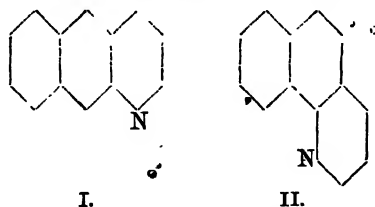
indamine and saffranine reactions when oxidised with aniline hydrochloride, toluylene red and blue colours with *m*-phenylenediamine, and methylene blue with hydrogen sulphide and ferric chloride.

Many other facts of a similar nature have been observed among derivatives of tri-cyclic systems.

Marckwald<sup>1</sup> sees in the different behaviour of amino-naphthalenes and amino-quinolines, when submitted to the reactions of Skraup and Doebner-Miller (pp. 277, 278), a confirmation of Kekulé's hypothesis, for according to the Erlenmeyer formula for naphthalene and the Körner formula for quinoline the positions 1.2 and 3.4 are different from 2.3, whilst in the centric formula they are equivalent.



With an amino group in position 1 or 2 the attachment of a pyridine nucleus by the above reactions never produces a compound of the symmetrical type (I), but always of the unsymmetrical type (II).



I.

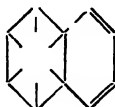
II.

On the other hand the dicentric formula is adopted by Willstätter<sup>2</sup>

<sup>1</sup> *Annalen*, 1893, 274. 331; 1894, 279, 14.

<sup>2</sup> *Ber.*, 1913, 40, 1051.

on the ground that whilst dihydronaphthalene is readily reduced by hydrogen in presence of platinum to the tetrahydro-compound, naphthalene behaves differently, inasmuch as no such intermediate reduction products can be isolated, but only forms the decahydro-compound, and consequently contains no ethenoid linkage. Stark also gives his support to this formula from the nature of the absorption bands, which closely resemble those of benzene (Part II, p. 87). Another formula has, however, been suggested, which lies midway between that of Erlenmeyer and the dicentric structure, and is known as Harries' centric-ethylene formula.



One benzene ring has the centric, the second the Kekulé arrangement. This view is chiefly based on the behaviour of naphthalene with ozone, with which it forms a diozonide instead of at least a tetra-ozonide as required by the Erlenmeyer formula (Part I, p. 121).

**General Properties of the Alkaloids.** From the long list of vegetable bases a few typical and better-known examples have been selected, with the object of illustrating the manner in which the resolution of the alkaloid has been effected, the constituent fragments of the molecule identified, and the problem of its structure finally solved. It is impossible to lay down other than broad generalizations in describing the alkaloids. They are confined to no special orders or parts of plants; but they are specially abundant in the families of Rubiaceae, Solanaceae, and Papaveraceae, and rare in those of Labiatae and Rosaceae. It is seldom that only one alkaloid is present in the plant, more commonly there are several; in opium, for example, as many as twenty individuals have been isolated, and the alkaloids which are associated in this way are usually closely related in structure and properties. Where the alkaloids are of a strongly poisonous character they are either elaborated by the protoplasm for defence against destruction by animals, or represent waste products which are stored in special cells and so rendered harmless to the plant. A few, which like conine and nicotine are liquid, are as a rule free from oxygen, but the majority are solids and contain oxygen. The larger number of alkaloids are colourless, but a few, such as berberine and sinapine,

have a yellow colour. They are very soluble in water, but are much more readily dissolved by alcohol, ether, and other organic solvents. They possess a bitter taste, an alkaline reaction, and form crystalline salts and double salts. The majority are optically active and these are nearly all laevogyrate. They have frequently been utilized for resolving inactive acids into their active components. They also exhibit characteristic absorption spectra (Part II, p. 77). They rarely exist in the plant in the free state, but are more frequently present as malates, citrates, lactates, tannates, or bound to some other acid which is a peculiar accompaniment of the alkaloid. Meconic acid, for example, is combined with morphine in opium, quinic acid with quinine, chelidonic acid with chelidonine, and aconitic acid with the alkaloids of aconitine. The alkaloids contain one or two atoms of nitrogen, rarely three or four, and the nitrogen imparts to the compound the function of a secondary, or more frequently of a tertiary, base. The nitrogen is firmly fixed in the molecule; but it can be occasionally removed as ammonia by the action of strong reducing agents; by the action of alkalis it is sometimes eliminated as methylamine, indicating the attachment of methyl to the nitrogen in the molecule. The number of such methyl groups can be ascertained by a method analogous to that of Zeisel for estimating methoxyl groups. The stability of the cyclic nitrogen atom in the alkaloid is greatly weakened by making the element quinquivalent. An example of this character has been given in the case of the benzyl chloride compound of quinoline, which on oxidation gives a derivative of anthranilic acid. This property has been utilized by Hofmann in breaking down some of the alkaloids and will be referred to later. When oxygen is present in the alkaloid it is usually in the form of hydroxyl or methoxyl, and occasionally as carboxyl or an ester group. The ordinary methods can be applied in determining the number and nature of these groups. The chemical characters of the alkaloids are very diverse. Some, like piperine, are amides; others, like atropine, are esters; solanine is a glucoside. In all these cases they can be hydrolysed. Cocaine on hydrolysis splits up into methyl alcohol, benzoic acid, and a base, *ecgonine*. Where hydrolysis can be effected, it always precedes any other process of decomposition. The action of alkalis, of zinc dust and of other reducing agents, has been used to effect further changes, but, of all reactions, the most valuable results have usually been derived from regulated oxidation. Among the numerous oxidising agents, potassium permanganate in acid or alkaline solution, nitric and chromic acids are generally employed. The alkaloids are

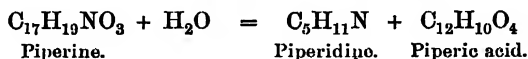
usually classified under (1) derivatives of pyridine; (2) derivatives of pyrrolidine; (3) derivatives of quinoline; (4) derivatives of isoquinoline; (5) derivatives of purine.

Examples are selected from each of these groups, excepting that of the purine bases, which is treated elsewhere (p. 102).

A very interesting theory of the mechanism of alkaloid synthesis in the plant is given by Robinson.<sup>1</sup>

## THE PYRIDINE ALKALOIDS

**Piperine.** The dried fruit of black pepper (*piper nigrum*) contains, in addition to a terpene and a resin, from 7–9 per cent. of a tasteless crystalline alkaloid termed piperine, which was discovered in 1819 by Oersted. Its composition,  $C_{17}H_{19}NO_3$ , was first correctly ascertained by Regnault. Piperine is a weak base, and is without action on polarized light. In 1845 Wertheim and Rochleder<sup>2</sup> found that, on distilling it with soda-lime, a volatile liquid passed into the receiver, possessing a strong ammoniacal smell and basic properties, which Cahours named *piperidine*, and gave it the formula  $C_5H_{11}N$ . A few years later Babo and Keller<sup>3</sup> discovered that piperine could be decomposed by means of alcoholic potash into piperidine and the potassium salt of a new acid, to which the name *piperic acid* was given. The decomposition may be represented as follows:



The result led to a partial synthesis by Rügheimer<sup>4</sup> in 1882, by acting on piperidine with piperic chloride dissolved in benzene,



from which it follows that piperine is probably an amide. Piperidine has a powerful ammoniacal smell, a strongly alkaline reaction, and exhibits all the properties of a secondary base.

The first important contribution to our knowledge of the constitution of piperidine is due to Hofmann,<sup>5</sup> who in 1879 showed that by heating it with bromine and water at 200–220°, dibromo-hydroxypyridine is formed. This close relationship to pyridine, from which piperidine differs by only six atoms of hydrogen, was further demon-

<sup>1</sup> *Trans. Chem. Soc.*, 1917, 111, 876.

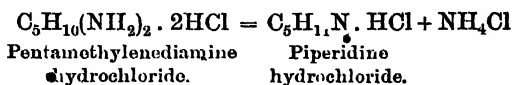
<sup>2</sup> *Annalen*, 1845, 54, 255.

<sup>3</sup> *J. prakt. Chem.*, 1857, 72, 53.

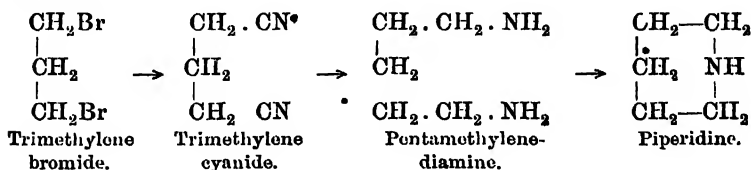
<sup>4</sup> *Ber.*, 1882, 15, 1890.

<sup>5</sup> *Ber.*, 1879, 12, 985.

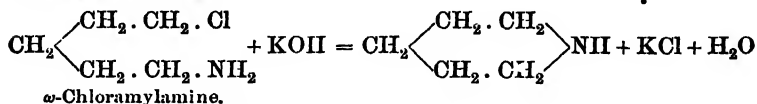
strated by Koenigs<sup>1</sup> in the same year, by converting it directly into pyridine by oxidation with strong sulphuric acid at 300°, and later, by Lellmann and Geller, who used nitrobenzene at a temperature of 250°. Piperidine must therefore be regarded as hexahydropyridine, a conclusion which was supported by the subsequent reduction of pyridine to piperidine by means of tin and hydrochloric acid, or by sodium and alcohol. Its complete synthesis was effected by Ladenburg<sup>2</sup> in 1885, by rapidly distilling the hydrochloride of pentamethylenediamine.



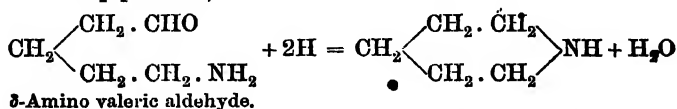
The latter compound is obtained from trimethylene bromide, which is successively converted into trimethylene cyanide, and on reduction with sodium in alcoholic solution into pentamethylenediamine.



Other syntheses have since been discovered. Gabriel and Blank converted  $\omega$ -chloramylamine and  $\omega$ -bromamylamine into piperidine by treating with alkali,<sup>3</sup>



and  $\delta$ -amino valeric aldehyde, which is obtained by the oxidation of benzoyl piperidine, can by the reverse process of reduction be transformed into piperidine,



It may be well to consider here some of the changes produced by the action of certain reagents on piperidine and its derivatives,

<sup>1</sup> Ber., 1879, 12, 2841.

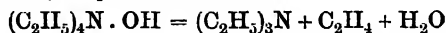
<sup>2</sup> Ber., 1885, 18, 2956, 3100.

<sup>3</sup> Ber., 1892, 25, 421, 3040.

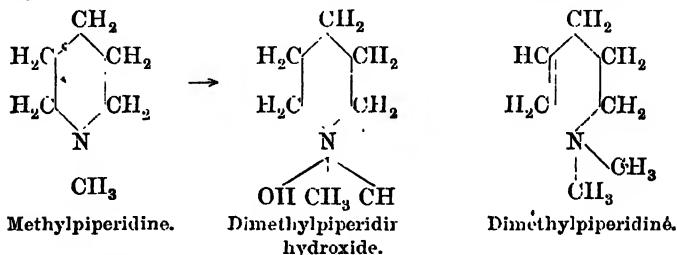


since similar reactions have been employed in the study of other alkaloids.

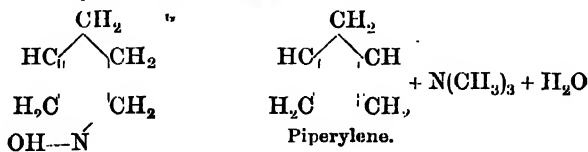
It has already been stated that when the nitrogen of the base becomes quinquivalent it is more subject to change. This was shown years ago by Hofmann<sup>1</sup> in the case of quaternary ammonium bases. Thus, tetraethylammonium hydroxide on heating breaks up into triethylamine, ethylene, and water.



In applying this reaction to piperidine, a similar change occurs.<sup>2</sup> Piperidine is convertible into the tertiary base, methyl piperidine, by the action of methyl iodide, and forms an additive compound with methyl iodide which yields in the ordinary way dimethylpiperidinium hydroxide. The latter, on distillation, loses water and forms a compound having the composition of dimethyl piperidine. These changes have been shown by Ladenburg to occur in the following way:



If now the last compound be in turn submitted to a similar series of reactions, the final product decomposes on distillation into piperylene, trimethylamine, and water.



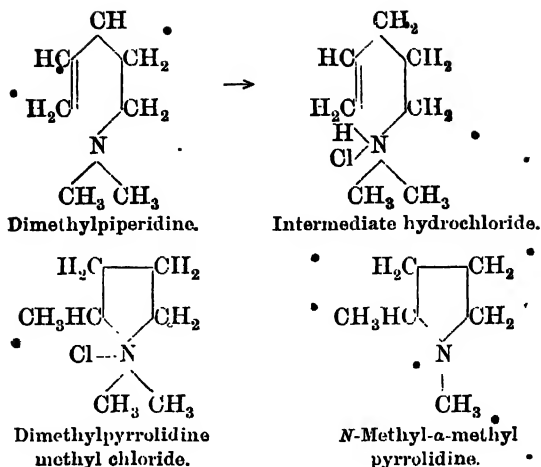
The process is known as 'exhaustive methylation'.

In connection with the open-chain, 'dimethylpiperidine,' Merling,<sup>3</sup> has made the interesting observation that on treatment with hydrochloric acid the chain closes, and by isomeric change a dimethyl pyrrolidine methyl chloride is formed, and at a higher temperature methyl chloride is removed and *N*-methyl- $\alpha$ -methyl pyrrolidine results.

<sup>1</sup> *Annalen*, 1851, 78, 263.

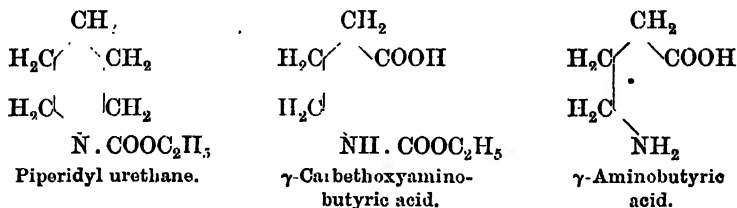
<sup>2</sup> *Ber.*, 1881, 14, 494, 659.

<sup>3</sup> *Annalen*, 1891, 264, 310.

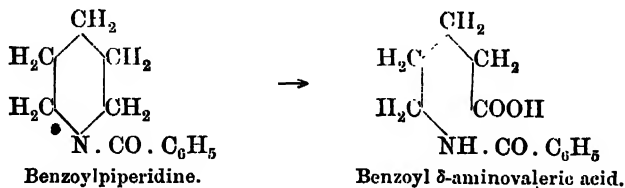


The presence of an acid radical attached to the nitrogen atom in place of hydrogen renders piperidine readily oxidisable, a process by which the ring is ruptured and an open-chain compound produced.

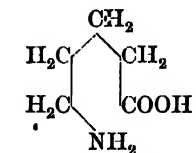
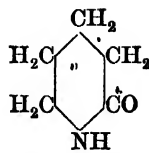
Thus, piperidyl urethane gives on oxidation  $\gamma$ -carbethoxyaminobutyric acid, which splits up with hydrochloric acid into ethyl chloride, carbon dioxide, and  $\gamma$ -aminobutyric acid,



whilst benzoyl piperidine on treatment with potassium permanganate forms benzoyl  $\delta$ -aminovaleric acid.



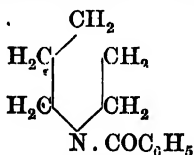
The latter process may be reversed, in the sense that, on heating,  $\delta$ -aminovaleric acid loses water and gives the lactam,  $\alpha$ -piperidone.

 $\delta$ -Aminovaleric acid. $\alpha$ -Piperidone.

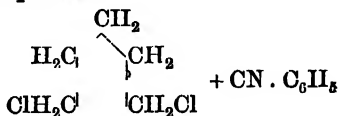
It is an interesting fact that, whereas the open-chain compound is without physiological action, piperidone is a strong poison.

The action of phosphorus pentachloride, which has been recently studied by v. Braun,<sup>1</sup> promises to become a useful addition to the methods of breaking down the cyclic structure in alkaloids.

Benzoylpiperidine with phosphorus pentachloride yields a mixture of benzonitrile and 1:5-dichloropentane.

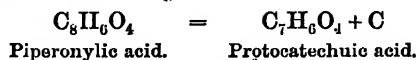


Benzoylpiperidine.

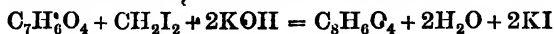


1:5-Dichloropentane. Benzonitrile.

**Pipecric Acid.** The acid constituent of piperine has still to be considered. Our knowledge of its structure is due to Fittig. Pipecric acid is a crystalline compound of the formula  $C_{12}H_{10}O_4$ . It is unsaturated, since it unites with four atoms of bromine, forming a tetrabromo derivative,  $C_{12}H_{10}Br_4O_4$ . On reduction with sodium amalgam it forms  $\alpha$ - and  $\beta$ -dihydropipecric acid,  $C_{12}H_{12}O_4$ , and the latter can take up two additional atoms of hydrogen, when the saturated tetrahydropipecric acid is obtained. On oxidation with potassium permanganate it yields two compounds, *piperonal*,  $C_8H_6O_3$ , and *piperonylic acid*,  $C_8H_6O_4$ . The former is an aldehyde which readily changes on oxidation into the latter. Piperonylic acid is a saturated acid and decomposes on heating with hydrochloric acid at  $170^\circ$ , or with water at  $210^\circ$ , into protocatechuic acid, carbon being separated.

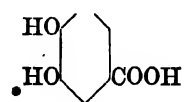


By reversal of the process, Fittig and Remsen succeeded in building up piperonylic acid. This was effected by heating protocatechuic acid with methylene iodide in presence of potash.

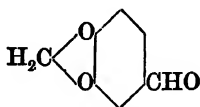


<sup>1</sup> Ber., 1904 37, 3583.

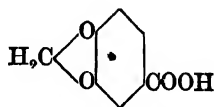
The structure of protocatechuic acid being known, the formulae of piperonal and piperonylic acid probably stand in the following relationship:



Protocatechuic acid.

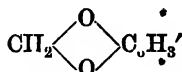


Piperonal.



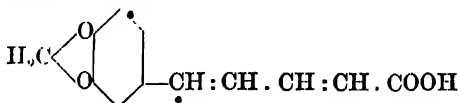
Piperonylic acid.

The group 'piperonyl'

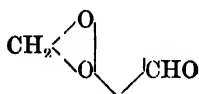


is not an uncommon constituent of vegetable products.

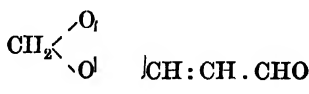
Piperic acid can only possess one side-chain corresponding to the carboxyl group of piperonylic acid, and since its formula is that of piperonylic acid with the addition of the di-olefinic group  $C_4H_4$ , it is probably represented as follows:



This agrees with the fact that it yields two isomeric dihydro derivatives, and also with Doebner's discovery that on careful oxidation it forms piperonal, the side-chain being oxidised to racemic acid. The structure of the acid is further confirmed by its synthesis from piperonal by Ladenburg and Scholtz.<sup>1</sup> Piperonal condenses with acetaldehyde in presence of caustic soda solution. The unsaturated aldehyde, piperonyl acrolein, is then converted by means of Perkin's reaction into piperic acid.



Piperonal.



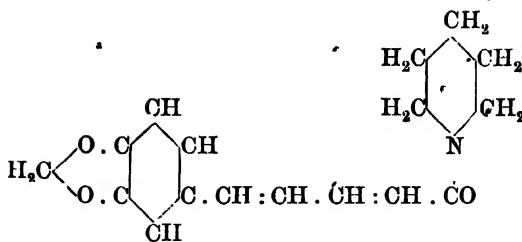
Piperonyl acrolein.



Piperic acid.

The complete structure of the alkaloid, piperine, is therefore represented by the following formula:

<sup>1</sup> Ber., 1894, 27, 2958.

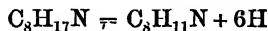


Piperine.

**Conine.** Hemlock (*conium maculatum*) contains the alkaloid conine,  $C_8H_{17}N$ , associated with smaller quantities of four other related substances,  $\gamma$ -coniceine,  $C_8H_{15}N$ , conhydrine,  $C_8H_{17}NO$ , the isomeric pseudoconhydrine, and *N*-methyl conine,  $C_9H_{19}N$ . It occurs in the plant in combination with malic and caffeic acids. The largest quantity (about 1 per cent.) is present in the unripe fruit, from which it may be obtained, along with the other bases present, by distillation with potash. It is a volatile oil with a penetrating and unpleasant smell, and is extremely poisonous. It is dextro-rotatory,  $[\alpha]_D = +18.3^\circ$ . Conine was discovered by Giesecke in 1827, but its composition was not accurately known until 1881, when Hofmann began his classical investigation, which ultimately disclosed the true constitution of this interesting alkaloid. In 1885 he pronounced it to be  *$\alpha$ -propyl piperidine*. The synthesis of the alkaloid—the first to be obtained artificially, was accomplished by Ladenburg in the year 1886.<sup>1</sup>

It would occupy too much space to do full justice to Hofmann's researches, which are contained in six papers published during the years 1881–5<sup>2</sup>; but it should be remembered that the path of inquiry which he pursued was then almost untrodden, and the obstacles which beset the pioneer in such an unknown region could only be surmounted by rare ingenuity and unfailing resource.

The constitution of conine is mainly based upon the behaviour of the hydrochloride with heated zinc dust. Contrary to the usual effect of this reagent, hydrogen is removed and conine forms a new base, *conyrrine*.



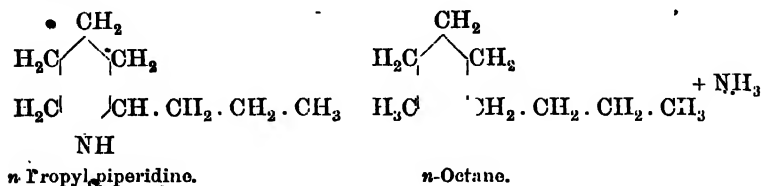
This compound yields picolinic acid on oxidation, and consequently contains a propyl or isopropyl side-chain in the  $\alpha$ -position. The choice between propyl and isopropyl pyridine is fixed by a comparison

<sup>1</sup> Ber., 1886, 19, 439, 2578. *Annalen*, 1888, 247, 1; 1894, 279, 34.

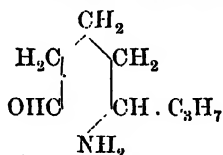
<sup>2</sup> Ber., 1881, 14, 705; 1882, 15, 2313; 1883, 16, 558; 1884, 17, 825, 1885, 18, 5, 109.

with the artificially prepared compounds and by the products of oxidation and reduction of conine itself.

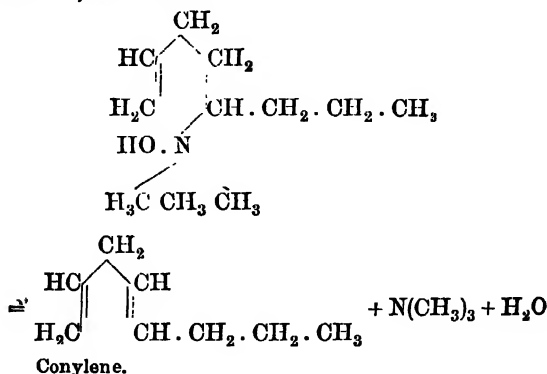
Strong hydriodic acid converts conine into ammonia and normal octane, which could only occur if the side-chain formed a normal linkage.



The oxidation products of the urethane and benzoyl derivatives of conine obtained by Schotten and also by Baum<sup>1</sup> which correspond to those of piperidine (p. 293) also indicate the presence of a normal propyl group, whilst by the use of hydrogen peroxide, Wolfenstein<sup>2</sup> obtained among other products amino-*n*-propyl valeric aldehyde.



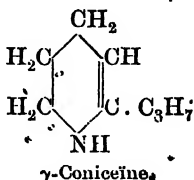
The evidence adduced by the process of exhaustive methylation points in the same direction, for on distilling the methyl hydroxide of dimethylconine, which is prepared like the corresponding compound of piperidine, it decomposes into a hydrocarbon *conylene*, trimethylamine, and water.



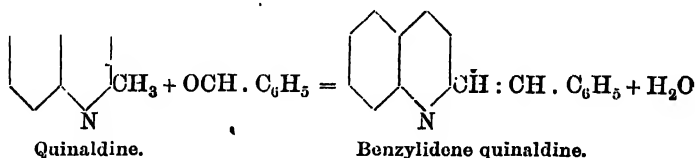
<sup>1</sup> Ber., 1882, 15, 1947; 1886, 19, 503.

<sup>2</sup> Ber., 1895, 28, 1460; 1904, 37, 3223.

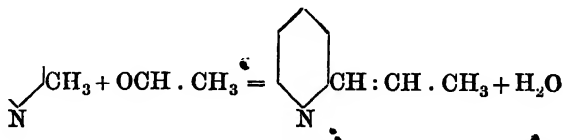
The substances described as *coniceïnes*, of which Hofmann prepared five isomers, are intermediate reduction products between conyryne and conine, and have the formula  $C_8H_{16}N$ .  $\gamma$ -Coniceïne, which is found in crude conine, can be prepared by the action of alkalis on chloro- or bromo-conine, and, since it is inactive, probably has the formula :



**Synthesis of Conine.** We have now to follow the steps by which Ladenburg\* with admirable perseverance succeeded in building up the artificial compound. The attempt to obtain a propyl pyridine by heating the propyl iodide compound of pyridine (Part II, p. 369) failed, because in the process an intramolecular change occurs, the normal propyl passing into the isopropyl group. The second method met with greater success. It was based upon an observation of Jacobsen and Reimer,<sup>1</sup> who found that when heated together quinaldine condenses with aldehydes and ketones to form alkylidene quinaldines. With benzaldehyde, benzylidene quinaldine is formed\*.

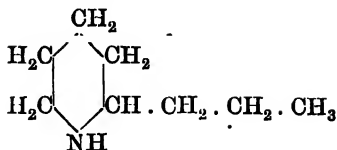
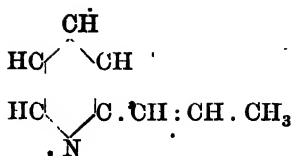


If  $\alpha$ -picoline is substituted for the base and acetaldehyde or paraldehyde for benzaldehyde, and the two heated to  $250^\circ$ , then a small yield of  $\alpha$ -allylpyridine is obtained.



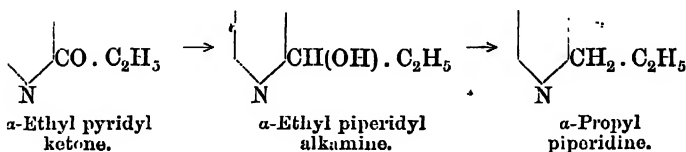
Allyl pyridine is reduced in alcoholic solution by sodium and converted into  $\alpha$ -propyl piperidine.

\* Ber., 1883, 16, 513, 1082, 1892, 2662, 2942.



The compound is nearly identical with the natural alkaloid; in one important property only is it lacking. The natural compound is dextrogyrate, whereas the artificial product is inactive. It is manifest that in propyl piperidine the carbon atom of the nucleus to which the side-chain is attached is asymmetric. (Part II). The artificial compound is probably the racemic form and capable of separation into two active constituents. This was the view taken by Ladenburg, and after many trials he succeeded in separating the dextro- and laevo-components by utilizing the different solubilities of the bitartrates. The dextro-base separated in this way has a higher rotation than the natural one, and is regarded as isoconine (Part II), but on heating gives the normal rotation, and proves in every respect identical with natural conine.<sup>1</sup>

A few years later Ladenburg's results were confirmed by a second synthesis by Engler and Bauer.<sup>2</sup> By distilling molecular equivalents of the calcium salts of propionic and picolinic acids, they obtained  $\alpha$ -ethyl pyridyl ketone, which on reduction is converted successively into  $\alpha$ -ethyl piperidyl alkamine and inactive  $\alpha$ -propyl piperidine.



## THE PYRROLIDINE ALKALOIDS

**Hygrine** was found by Liebermann<sup>3</sup> in 1889 among the alkaloids of coca, in which it is present in minute quantities. It is a liquid, b.p. 193–195°, and is laevo-rotatory. It is a tertiary base containing a N-methyl group, and is a ketone, since it forms an oxime. On oxidation it yields a monobasic acid, *hygric acid*,  $\text{C}_5\text{H}_{11}\text{N} \cdot \text{COOH}$ :

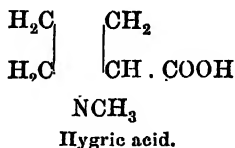
<sup>1</sup> Ber., 1906, 39, 2486

<sup>2</sup> Ber., 1891, 24, 2530; 1894, 27, 1775.

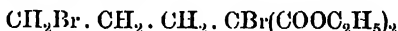
<sup>3</sup> Ber., 1891, 24, 407; 1895, 28, 578.



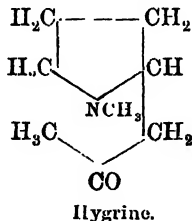
which on dry distillation decomposes into  $\text{CO}_2$  and N-methyl pyrrolidine. Hygric acid must therefore have the structure,



the position of the carboxyl group being determined by its easy removal on heating. The acid has since been synthesised by Willstätter and Ettlinger<sup>1</sup> by the action of methylamine on  $\alpha\delta$ -dibromopropylmalonic ester.



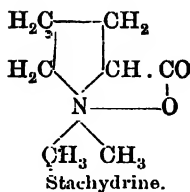
As hygrine differs from hygric acid by the group  $\text{C}_3\text{H}_5\text{O}$  in place of carboxyl, and as this group contains a ketone group, the side-chain may be  $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CO}$  or  $\text{CH}_3 \cdot \text{CO} \cdot \text{CH}_2$ . From the fact that tropinone (p. 307) contains the second of the alternative arrangements, it is probable that the same group is present in hygrine, which will therefore have the formula:



**Stachydrine.** Another of the simple pyrrolidine alkaloids is *stachydrine*,  $\text{C}_7\text{H}_{13}\text{NO}_2 + \text{H}_2\text{O}$ , which is found in the root-nodules of *stachys tubifera*, and in some other plants. It was discovered by v. Planta and Schulze in 1893, who pointed out that it was probably a betaine, from its strong resemblance to trigonelline (p. 271). It crystallizes in colourless, deliquescent crystals. On destructive distillation the vapours give the reaction for pyrrole. It contains a carboxyl group, and when heated with concentrated potassium hydroxide evolves dimethylamine. From these facts Schultze and Trier<sup>2</sup> suggested that the structure was that of a dimethyl betaine of  $\alpha$ -proline.

<sup>1</sup> *Annalen*, 1903, 326, 91.

<sup>2</sup> *Zeit. physiol. Chem.*, 1909, 59, 233.



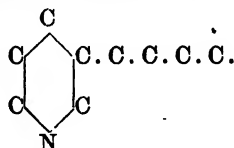
In the same year they confirmed their view by effecting its synthesis from hygric acid (see above).<sup>1</sup> The ester of this acid combines with methyl iodide to form a quaternary iodide; the iodine was then replaced by the action of silver oxide and gave stachydrine.

**Nicotine** is the alkaloid of tobacco leaves, and, like conine, it is associated with smaller quantities of at least three other, probably similarly constructed, substances. The alkaloid is found in the plant in combination with malic and citric acid, and the quantity varies between 0.6–8 per cent., the average being about 4 per cent., good leaves containing less than inferior specimens. It is an oil, which boils at 247°, and is a powerful poison, even in the form of vapour. It is laevogyrate. It was originally discovered by Posselt and Reimann in 1828, but its true formula ( $\text{C}_{10}\text{H}_{14}\text{N}_2$ ) was first ascertained by Melsens. Nicotine has been the subject of prolonged and careful study, chiefly by Blau and Pinner, and later by Pictet, who, in 1904, in conjunction with Rotschy, succeeded in preparing it artificially, thereby confirming the structural formula arrived at by Pinner's researches. We propose to follow the principal steps which have led to the knowledge of the structure and ultimately to the synthesis of nicotine, omitting those reactions which have a less important bearing on the problem before us.

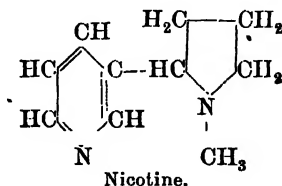
Nicotine is a di-acid and also a bi-tertiary base, for it unites with two molecules of alkyl iodide, and likewise forms two different quaternary compounds containing one molecule of methyl iodide. Of the latter, one is obtained by the action of methyl iodide on the hydriodide of the base. When transformed into the quaternary hydroxide and oxidised, Pictet and Genequand obtained trigonelline (p. 271), from which it follows that pyridine is of the two the weaker basic group. The stronger oxidising agents convert nicotine into nicotinic acid, that is,  $\beta$ -pyridine carboxylic acid. Milder oxidising agents like silver oxide or potassium ferricyanide yield *nicotyrine*  $\text{C}_{10}\text{H}_{10}\text{N}_2$ , whilst hydrogen peroxide gives a substance  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{O}$  of unknown constitution which is named *oxynicotine*. Thus far, we may conclude that one basic group is pyridine, and that it is

<sup>1</sup> Ber., 1909, 42, 4651.

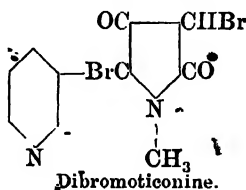
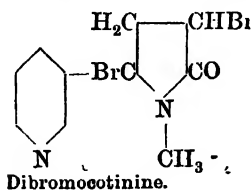
linked in the  $\beta$ -position to the second basic group. The nature of this second group is mainly derived from Pinner's researches on the bromination of nicotine, which will now be considered. At ordinary temperatures nicotine gives a perbromide,  $C_{10}H_{11}Br_3N_2O$ , from which water or ammonia easily removes three of the bromine atoms (one as hydrogen bromide), forming *dibromocotinine*  $C_{10}H_{10}Br_2N_2O$ . By the action of bromine at  $100^\circ$  the hydrobromide of *dibromoticonine*  $C_{10}H_8Br_2N_2O_2$  is formed. Both compounds are decomposed by bases; the first breaks up into methylamine, oxalic acid, and the base  $C_7H_7NO$ , which is probably  $\beta$ -methyl pyridyl ketone, the second forms methylamine, malonic acid and nicotinic acids. These facts afford valuable information. The second basic group contains one carbon atom as methyl, united to a nitrogen atom. The simultaneous appearance of malonic and nicotinic acid among the fragments of bromoticonine imply a  $\beta$ -pyridine side-chain of four carbon atoms, and hence will possess a skeleton structure of the following character:



Seeing that nicotine is a bitertiary base, Pinner concluded that the atoms of the second basic group were fused into a methyl pyrrolidine nucleus thus:

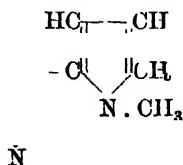


Nicotine therefore represents a  $\beta$ -pyridyl- $\alpha$ -*N*-methylpyrrolidine. Let us examine in the light of this formula the behaviour of nicotine on bromination. According to Pinner, dibromocotinine and dibromoticonine are probably represented by the following formulae:



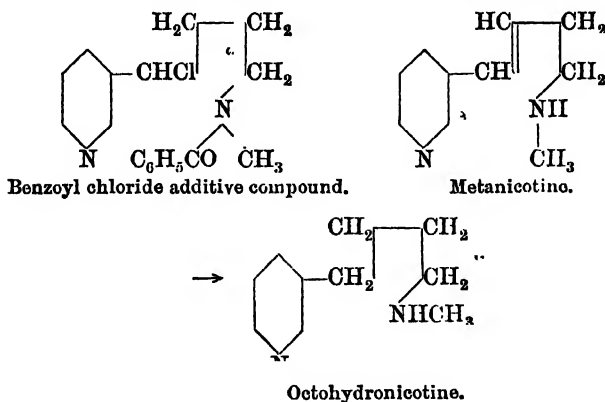
The action of bases on these two substances would be to replace bromine by hydroxyl. This would be followed by a rupture of the pyrrolidine nucleus, and the formation of pyridyl methyl ketone and oxalic acid in the first case and of nicotinic and malonic acid in the second.

The other properties of nicotine are also easily explained. Nicotyrine, which contains 4 hydrogen atoms less than nicotine, will probably be represented by the following formula :



Nicotyrine.

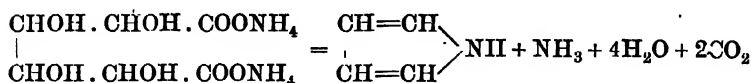
whilst hexahydronicotine, which is obtained by the reduction of nicotine with sodium in alcohol solution, will correspond to the piperidine derivative. The production of the octohydronicotine, which is also formed on reduction, can only be explained by the rupture of the pyrrolidine nucleus. This is confirmed by the following fact. The additive compound of nicotine with benzoylchloride is converted by sodium alcoholate into *metanicotine*, a base isomeric with nicotine which on reduction gives octohydronicotine. The explanation is denoted by the following formulae :



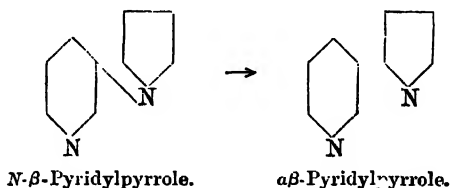
**Synthesis of Nicotine.** The merit of this achievement belongs to Pictet, Crépieux, and Rotschy.<sup>1</sup> The steps in the discovery are

<sup>1</sup> *Ber.*, 1895, 28, 1901; 1904, 37, 1225.

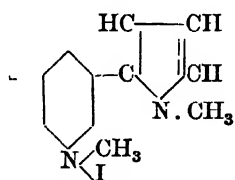
briefly as follows: Pictet and Crépieux obtained *N*- $\beta$ -pyridyl pyrrole by the distillation of  $\beta$ -aminopyridine mucate, a reaction which corresponds exactly to the formation of pyrrole from ammonium mucate.



According to an observation of Ciamician the *N*-alkyl derivatives of pyrrole like those of pyridine undergo molecular change on heating by the shifting of the radical from the nitrogen to the  $\alpha$ -carbon. By passing the vapour of *N*-pyridylpyrrole through a red-hot tube it isomerises to  $\alpha\beta$ -pyridylpyrrole.

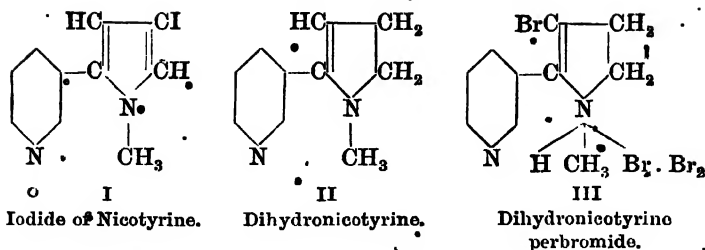


By the action of methyl iodide on the potassium salt of the latter  $\alpha\beta$ -pyridyl-*N*-methylpyrrole methiodide is formed, which is identical with nicotyrine methiodide.



Nicotyrine methiodide.

As nicotine contains four atoms of hydrogen more than nicotyrine, which may be regarded as its first oxidation product, the next problem was to reduce nicotyrine. This cannot be effected directly; but by the action of iodine and caustic soda on nicotyrine from natural nicotine a crystalline iodine substitution product is obtained (I), which can be reduced with zinc and hydrochloric acid to dihydronicotyrine (II). The last two hydrogen atoms can be introduced by the reduction of the perbromide of dihydronicotyrine (III).



The new base appeared to be identical with inactive nicotine. The final problem was how to isolate the artificial nicotyrine from its methiodide. After several unsuccessful trials this was eventually accomplished by distilling it with lime at as low a temperature as possible. Inactive nicotine was then prepared from the product in the manner described above and resolved like inactive conine into its active components by crystallizing the tartrates. A comparison of synthetic *l*-nicotine with the natural alkaloid showed complete identity. The difference in the physiological action between the *d*- and *l*-nicotines is remarkable and has been referred to already in Part II, p. 182.

**Atropine.** Some of the Solanaceae—deadly night-shade or belladonna (*atropa belladonna*), henbane (*hyoscyamus niger*), thorn-apple (*datura stramonium*) and an Australian plant, *duboisia myoporoides*—contain several alkaloids which are closely related in chemical and physiological properties. Four of these, *atropine*, *hyoscyamine*, *ψ-hyoscyamine* and *hyoscyne* are isomeric, and have the formula  $C_{17}H_{23}NO_3$ ; the others, *belladonnine*, *apoptropine*  $C_{17}H_{21}NO_2$  and *scopolamine*  $C_{17}H_{21}NO_4$ , are little known. The most important are atropine and hyoscyamine, which are present in all the plants named and in all parts of the plant, though the total quantity is small and rarely exceeds one-half per cent.

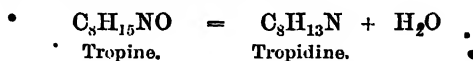
Atropine and hyoscyamine, to which we shall confine our attention, are usually extracted from the root of the deadly night-shade with alcohol, from which, after precipitating the colouring matter and removing the alcohol by distillation, the bases are set free with potassium hydroxide and extracted with chloroform.

It may be stated at once that hyoscyamine is the laevo modification of atropine which represents the inactive racemic form, so that in the description of the general chemical properties and structure both compounds are included.

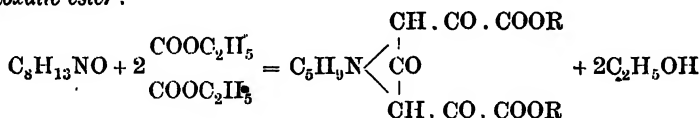
Atropine is a crystalline compound which partly sublimes on heating. It is extremely poisonous and is optically inactive. It



obtained by Ladenburg by heating tropine with hydrochloric acid at 180°, with dilute sulphuric acid at 220°, or with potassium hydroxide, whereby a molecule of water is removed.



It is also formed by the action of hydriodic acid and phosphorus at 140°. It is an unsaturated compound uniting with one molecule of hydrogen, the halides and halide acids. The above facts point to the existence of a *hydroxyl group* in tropine. That the group is present in the form of a *secondary alcohol*, is shown by the oxidation of tropine to the ketone *tropinone*  $\text{C}_8\text{H}_{13}\text{NO}$ . Reduction of tropinone does not, however, regenerate tropine, but the stereoisomer, *ψ-tropine*, which is identical with a product obtained from an alkaloid associated with cocaine. Protracted oxidation with chromic acid converts tropinone into the dibasic *tropinic acid*,  $\text{C}_6\text{H}_{11}\text{N}(\text{COOH})_2$ , which is also obtained by the action of permanganate on tropidine. Furthermore, the ketone group of tropinone is situated between two methylene groups; for the reactions of the complex,  $\text{CH}_2 \cdot \text{CO} \cdot \text{CH}_2$ , are characteristic, and are consistent with the behaviour of tropinone.<sup>1</sup> For example, tropinone undergoes condensation with two molecules of benzaldehyde, forming a dibenzylidene compound; with two molecules of oxalic ester in presence of sodium ethoxide, giving *tropinone-dioxalic ester* :



with amyl nitrite in presence of acetic and hydrochloric acid, yielding *diisonitrosotropinone*. Finally, tropinone combines with two molecules of diazobenzene.

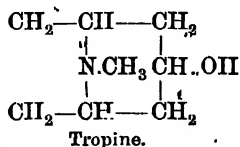
The existence of a pyridine, or more accurately piperidine, nucleus in tropine has been demonstrated by Ladenburg<sup>2</sup> in various ways. He heated tropidine hydrobromide with bromine to 170–180° and obtained *α-methyl dibromopyridine*, and also by using an excess of bromine, Hofmann's *dibromopyridine*.<sup>3</sup> Further, he found that tropidine on reduction is converted into *hydrotropidine*,  $\text{C}_8\text{H}_{16}\text{N}$ , which breaks up when heated in a current of hydrogen chloride into *norhydrotropidine* and methyl chloride. When distilled over zinc dust, the latter loses hydrogen and yields *α-ethyl pyridine*. Let us

<sup>1</sup> Willstätter, *Ber.*, 1897, **30**, 2679.

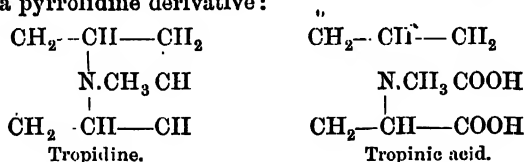
<sup>2</sup> *Annalen*, 1883, **217**, 141.



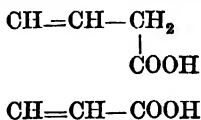
now review the evidences of structure which the above facts afford. The formula for tropine,  $C_8H_{15}NO$ , includes a tertiary *N*-methyl pyridine nucleus,  $CH_3NC$ , and the group,  $CH_2 \cdot CH(OH) \cdot CH_2$ . Without discussing the views of Ladenburg and Merling, which rested on the incomplete knowledge of earlier researches, we will pass at once to the consideration of Willstätter's formula, which is the result not only of a more extended inquiry into the products of disintegration of tropine, but of the more convincing evidence afforded by its subsequent synthesis:



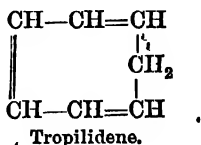
According to this formula, tropine not only contains a hydroxy-*N*-methylpiperidine, but also a pyrrolidine nucleus, as well as a carbon ring of seven atoms. Have we any evidence of the presence of these two nuclei? It seems not improbable that tropinic acid, the product of oxidation of tropidine, is represented by the formula below, which is that of a pyrrolidine derivative:



For by Hofmann's method of exhaustive methylation applied to tropinic acid, by which nitrogen is removed as trimethylamine, a diolefinic dibasic acid,  $C_6H_8(COOH)_2$ , results, which on reduction yields normal pimelic acid, and has undoubtedly the following structure:



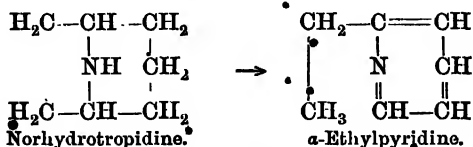
The cycloheptane ring is probably represented by tropilidene,  $C_7H_8$ , which is obtained by the exhaustive methylation of tropine and tropidine (p. 306).



The fact that tropilidene gives benzaldehyde on oxidation, and

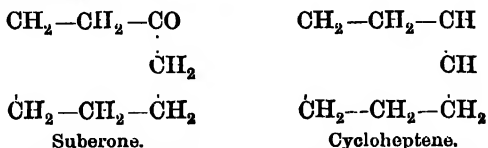
forms a dibromide which, when heated, decomposes into hydrobromic acid and benzyl bromide, led Merling to regard tropolidene as a benzene derivative; but the conversion of a seven-ring into a six-ring complex is not by any means uncommon, and may very well occur in the present instance.

The formation of norhydrotropidine and its conversion into  $\alpha$ -ethylpyridine is very simply represented by means of the following formulae:

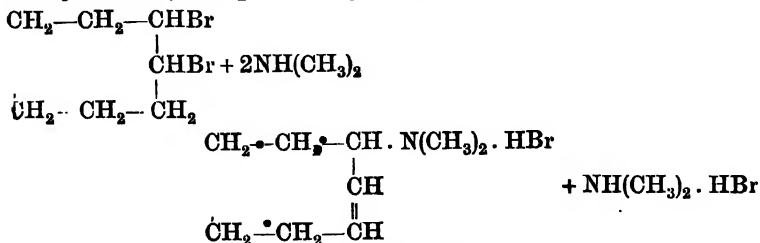


In this way the various phases in the resolution of the tropine molecule have been satisfactorily explained.

**Synthesis of Tropine.**<sup>1</sup> The starting-point in the synthesis of tropine is suberone, a cycloheptanone which was originally obtained by the distillation of the calcium salt of suberic acid. This is converted successively into the alcohol and into the iodide. By the removal of hydrogen iodide with alcoholic potash from the latter, cycloheptene is obtained.

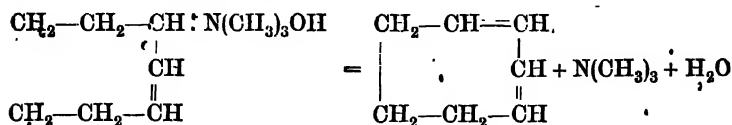


Another method for obtaining cycloheptene is to reduce suberone-oxime to suberylamine and apply the method of exhaustive methylation. The introduction of a second double linkage into the nucleus is attended with greater difficulty, and can only be accomplished by an indirect method. Cycloheptene dibromide is acted upon with dimethylamine, whereby an unsaturated base,  $\Delta^2$ -dimethylamine cycloheptene, is produced.

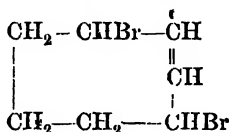


<sup>1</sup> Willstätter, *Annalen*, 1901, 317, 204.

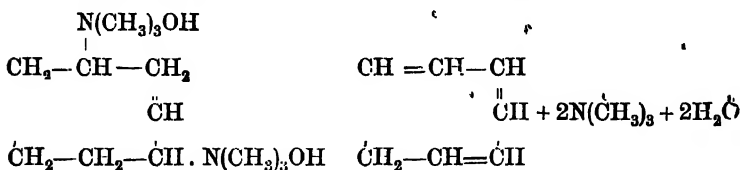
The latter is converted into the quaternary ammonium iodide and hydroxide, and then distilled, when it breaks up into trimethylamine, cycloheptadiene, and water.



Cycloheptadiene contains a conjugated system of double bonds (Part I, p. 132), and with one molecule of bromine forms a dibromide, from which cycloheptatriene can be obtained in two ways



Dimethylamine combines to form a di-acid base which, by exhaustive methylation, gives cycloheptatriene as follows:

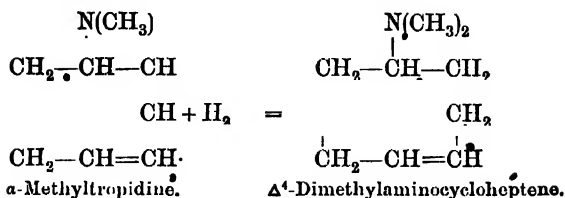


Or hydrogen bromide may be directly eliminated by boiling with quinoline.

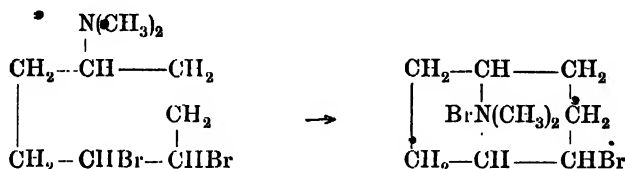


The cycloheptatriene obtained in this way is identical in every respect with Ladenburg's tropilidene (p. 306).

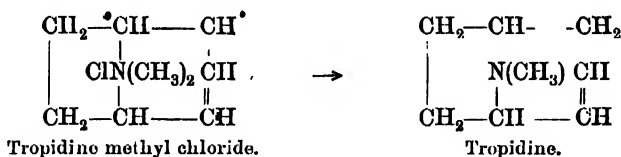
The next step in the synthesis consists in the conversion of tropilidene into tropidine. Cycloheptatriene forms a monohydrobromide with hydrogen bromide in the cold, and the hydrobromide reacts with dimethylamine, giving a product which is identical with  $\alpha$ -methyltropidine, obtained by distilling tropidine methyl ammonium hydroxide. This is converted into  $\Delta^1$ -dimethylaminocycloheptene on reduction.



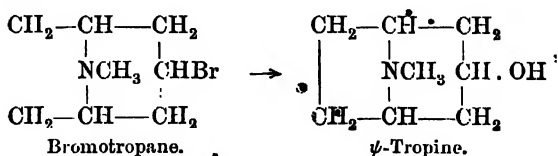
$\Delta^4$ -Dimethylaminocycloheptene unites with a molecule of bromine in the cold, which, on warming, rapidly isomerises to bromotropane-methylammonium bromide.<sup>1</sup>



Potassium hydroxide readily removes hydrobromic acid from the latter and converts it into tropidine methylammonium bromide.<sup>2</sup> From the corresponding chloride, methyl chloride may be removed and tropidine itself obtained by distillation.



The conversion of tropidine into  $\psi$ -tropine was effected by Willstätter<sup>2</sup> in the following way: Tropidine was combined with hydrogen bromide and heated with sulphuric acid in a closed tube to 200°.



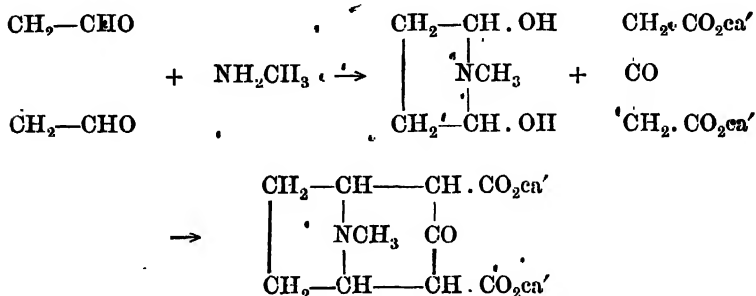
The conversion of  $\psi$ -tropine into its stereoisomer tropine was accomplished by oxidising  $\psi$ -tropine to tropinone and reducing the

<sup>1</sup> Tropane is the name given to the simple saturated bicyclic complex.

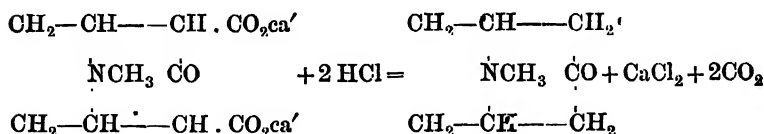
<sup>2</sup> Ber., 1900, 33, 1170.

ketone with zinc dust and hydriodic acid. Ladenburg<sup>1</sup> has also transformed tropidine into tropine directly by the action of hydrogen bromide.

A more recent and simpler synthesis by way of tropinone is described by Robinson.<sup>2</sup> It consists in mixing succindialdehyde, methylamine and either the calcium salt or ester of acetone dicarboxylic acid, when the following condensation occurs,



The latter on heating with acid gives tropinone, carbon dioxide being eliminated:



**Structure and Synthesis of Tropic Acid.** It has already been stated that tropic acid is formed with tropine when atropine is hydrolysed. Kraut found that it gave benzoic acid on oxidation, and when fused with potassium hydroxide breaks up into phenylacetic and formic acids. When treated with phosphorus pentachloride, tropic acid exchanges two hydroxyl groups for chlorine,

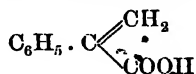


and when heated to 160° it loses a molecule of water and forms the lactone *tropide*,  $\text{C}_9\text{H}_8\text{O}_2$ , which is converted into the isomeric *atropic*

<sup>1</sup> Ber., 1890, 23, 1780, 2225; 1902, 35, 1159, 2295.

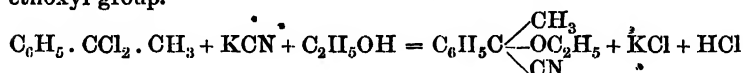
<sup>2</sup> Trans. Chem. Soc., 1917, 111, 762.

acid on boiling with baryta water. That atropic acid is a benzene derivative with one side-chain follows from the fact that it yields benzoic acid on oxidation. Moreover, it is unsaturated, forming additive compounds with one molecule of hydrogen bromide or bromine. It is therefore closely related to cinnamic acid, with which it is in fact isomeric, and is probably represented by the formula,

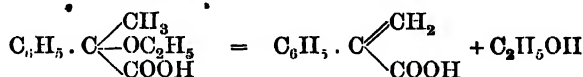


This structure has been confirmed by its synthesis by Ladenburg and Rügheimer<sup>1</sup> in 1880.

Acetophenone is converted into the dichloride by the action of phosphorus pentachloride. When the dichloride is treated with an alcoholic solution of potassium cyanide, a double reaction occurs, one chlorine atom being replaced by cyanogen and the other by the ethoxyl group.



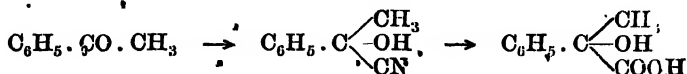
The product being a cyanide can be hydrolysed, and the resulting ethyl  $\alpha$ -phenyllactic acid, when heated with strong hydrochloric acid, loses a molecule of alcohol and yields atropic acid



Atropic acid is clearly the anhydride of tropic acid. By the addition of the elements of water the hydroxyl may attach itself to the end or middle carbon atom of the side-chain. Two isomers must therefore exist of the following formulae:



An acid having the first of these formulae has long been known under the name of *atrolactic acid*. It was obtained originally by Fittig and Wurster by heating with sodium carbonate the hydrogen bromide additive compound of atropic acid. Later it was synthesized and its structure ascertained by Spiegel<sup>2</sup> by the simple process of hydrolysing the cyanhydrin of acetophenone.

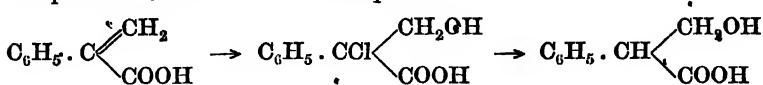


<sup>1</sup> Ber., 1880, 13, 373, 2041; see also E. Müller, Ber., 1918, 51, 252.

<sup>2</sup> Ber., 1881, 14, 1853.

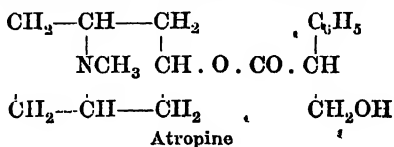
Consequently the second of the two formulae must be that of tropic acid. This view has been fully confirmed by its synthesis from atropic acid.

Atropic acid unites with hypochlorous acid, and the additive compound on reduction forms tropic acid.



According to this formula, tropic acid contains an asymmetric carbon, and it has been resolved into its two enantiomorphs by crystallizing the quinine salt.

**Structure of Atropine.** The fact that the hydrolysis of atropine produces tropine which possesses alcoholic functions and an acid, tropic acid, shows that the alkaloid itself must be an ester. This structure finds expression in the following formula:



**Hyoscyamine**, according to the researches of Gadamer,<sup>1</sup> is the ester of tropine and *l*-tropic acid; Hesse and Ladenburg have shown that *apoa tropine* (*atropamine*) is the tropeine of atropic acid, which undergoes isomerisation into *belladonine* on heating, whilst, according to Liebermann, the coca alkaloid, *tropacocaine*<sup>2</sup> (see below), is the benzoic ester of  $\psi$ -tropine. All these substances must now be included in the list of synthetic products.  $\psi$ -*Hyoscyamine* differs only from hyoscyamine in having a hydrogen atom in place of methyl attached to the nitrogen atom of the bridge.<sup>3</sup>

**Hyosine** (*Scopolamine*) is the trotyl ester of *oscine* (*scopoline*) which has been synthesized by Hess and Merck.<sup>4</sup>

**Cocaine.** The leaves of *erythroxylon coca* contain a series of alkaloids, among which *l*-cocaine, *d*-cocaine, *tropa-cocaine*, *cinnamyl-cocaine*,  $\alpha$ - and  $\beta$ -*truxilline* and *hygrine* have been identified as distinct constituents. *l*-Cocaine, the most abundant and physiologically the most valuable constituent (it acts as a local anaesthetic), was isolated by Niemann as far back as 1860, and its formula,  $\text{C}_{17}\text{H}_{21}\text{NO}_4$ , was ascertained by Lossen. In recent years the study of the alkaloid

<sup>1</sup> *Arch. d. Pharm.*, 1902, 239, 294.

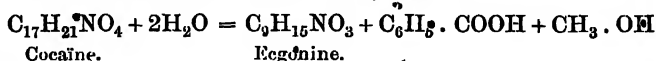
<sup>2</sup> Liebermann, *Ber.*, 1891, 24, 2336.

<sup>3</sup> Carr and Reynolds, *Trans. Chem. Soc.*, 1912, 101, 946.

<sup>4</sup> *Ber.*, 1919, 52, 1976; King, *Trans. Chem. Soc.*, 1919, 115, 476.

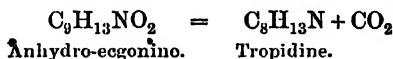
has been pursued with remarkable success by Einhorn, Liebermann; and Willstätter. Its close relationship to atropine has been demonstrated in various ways, and its structure has been finally established by its synthetic preparation.

*l*-Cocaine, like atropine, is a crystalline, tertiary base, and also, like atropine, it is an ester which, on hydrolysis with mineral acids or baryta, breaks up into a new tertiary base, *l*-ecgonine, benzoic acid and methyl alcohol, according to the equation:

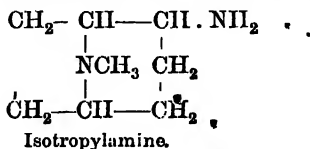


By a reversal of the process, *l*-cocaine has been reconstructed from *l*-ecgonine.<sup>1</sup> The structure of cocaine, therefore, depends upon that of ecgonine. The various cacaïnes, natural and artificial, are esters of ecgonine with different acids. In cinnamyl cocaine, ecgonine is combined with cinnamic acid, in truxilline with truxillic acid, and so forth.

Einhorn<sup>2</sup> has shown that ecgonine, like tropine, loses water with dehydrating agents such as hydrochloric and sulphuric acid, and the *anhydro-ecgonine*,  $\text{C}_9\text{H}_{13}\text{NO}_2$ , so formed, when heated to  $280^\circ$  with hydrochloric acid, is converted into tropidine.



This fact contains the key to the problem. Anhydro-ecgonine is obviously tropidine carboxylic acid, whilst ecgonine itself is in all probability tropine carboxylic acid. The positions of the hydroxyl and carboxyl groups have still to be ascertained. Anhydro-ecgonine was reduced by Willstätter to the dihydro derivative or *hydroecgonidine*, and the carboxyl was then replaced by the amino group, either by Hofmann's method through the amide or by that of Curtius by way of the hydrazide, azide and urethane.<sup>3</sup>



The product was distinct from either of the geometrical isomeric trotylamines obtained from tropine and  $\psi$ -tropine, and was termed

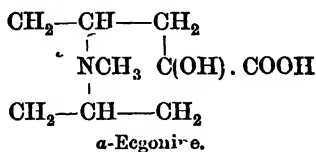
<sup>1</sup> Merck, *Ber.*, 1885, 18, 2952; Liebermann, *Ber.*, 1888, 21, 3196; 1894, 27, 2051.

<sup>2</sup> *Ber.*, 1889, 22, 399; 1890, 23, 1338.

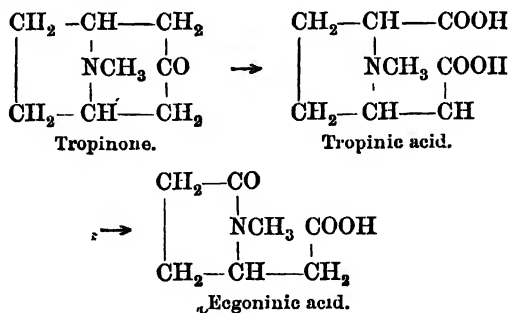
<sup>3</sup> *Ber.*, 1896, 29, 782.



*isotropylamine*. It follows, therefore, that the carboxyl group does not occupy the position of the hydroxyl in tropine. Furthermore, the hydroxyl group in ecgonine is not attached to the same carbon atom as the carboxyl, for tropinone cyanhydrin gives on hydrolysis a compound similar to, but distinct from, ecgonine, which is therefore named  $\alpha$ -ecgonine, giving an  $\alpha$ -methyl benzoyl ester known as  $\alpha$ -cocaïne.



This is also confirmed by the fact that ecgonine on oxidation with chromic acid gives a ketonic acid, which could only occur if the carboxyl and hydroxyl were attached to different carbon atoms. But there still remains a choice between the  $\beta$ - and  $\gamma$ -positions for the hydroxyl group. There are several facts which favour the  $\beta$ -position, and these are furnished by the products of oxidation. Ecgonine, by careful oxidation with chromic acid, loses carbon dioxide and is converted into tropinone. More energetic oxidation produces tropinic acid (p. 308) and ecgoninic acid. The tropinic acid only differs from the acid from tropine by being optically active (dextrogyrate), whilst the structure of ecgoninic acid is known from its synthesis by Willstätter and Hollander.<sup>1</sup>

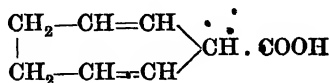


It is clear that the hydroxyl stands in the  $\beta$ -position to the carboxyl group; but if any further doubt existed on the subject, it has been set at rest by the synthesis of *r*-cocaïne from tropinone by Willstätter<sup>2</sup>.

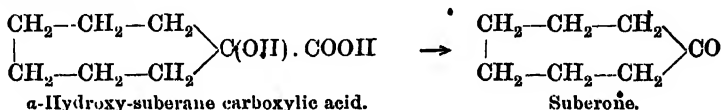
<sup>1</sup> *Annalen*, 1903, 326, 79.

<sup>2</sup> *Ber.*, 1900, 33, 411; *Annalen*, 1903, 326, 42.

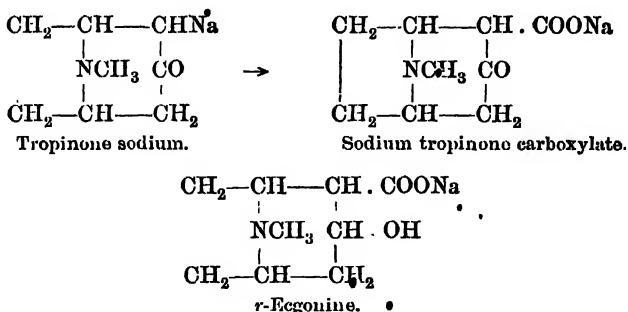
Before referring to the synthesis, it may be of interest to point out that just as tropine furnishes a cycloheptane ring in the form of tropilidene (p. 308), so ecgonidine or, more strictly, hydroecgonidine may be resolved into a cycloheptadiene carboxylic acid by exhaustive methylation and ultimately into suberone. The ethyl ester of hydroecgonidine is first converted into the nitrogen free acid, cycloheptadiene carboxylic ester.



The latter is reduced to the saturated cycloheptane carboxylic acid. Bromine is then introduced in the  $\alpha$ -position and replaced by hydroxyl. The hydroxy-acid, when submitted to oxidation with lead peroxide, yields suberone.

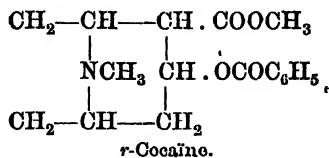


**Synthesis of *r*-Cocaine.** Tropinone sodium, when suspended in ether and treated with carbon dioxide, forms sodium tropinone-carboxylate, and the latter, on reduction with sodium amalgam in faintly acid solution, is converted into *r*-ecgonine and a second isomer.



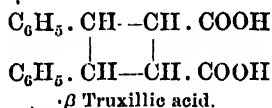
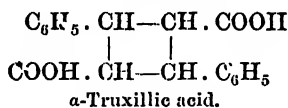
The *r*-ecgonine differs in optical properties from the ecgonine of the vegetable alkaloids; for naturally it is inactive, and, as it contains four asymmetric carbon atoms, may represent several pairs of enantiomorphs. Apart from this, it closely resembles *l*-ecgonine, and, like it, may be converted into the methyl benzoyl ester or *r*-cocaine.<sup>1</sup>

<sup>1</sup> See v. Braun and Müller, *Ber.*, 1918, 51, 235.

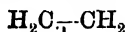
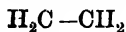


The second isomeric ecgonine which forms the greater portion of the reduction product is probably a  $\psi$ -tropine *O*-carboxylic acid; for it contains no hydroxyl group and resists the ordinary process of methylation.<sup>1</sup>

Of the substances which accompany cocaine in coca leaves, cinnamyl cocaine contains cinnamyl in place of benzoyl;  $\alpha$ - and  $\beta$ -truxilline contain in the same way the radicals of  $\alpha$ - and  $\beta$ -truxillic acid or dicinnamic acids.



Tropacocaine is the benzoic ester of  $\psi$ -tropine, whilst hygrine is probably the methyl ketone of *N*-methyl  $\alpha$ -methyl pyrrolidine, as



Hygrine.



Hygric acid.

determined by Willstätter's synthesis of hygric acid<sup>2</sup> and Hess's synthesis of hygrine (p. 299).<sup>3</sup>

**Pelletierine**,  $\text{C}_8\text{H}_{15}\text{NO}$ , occurs together with the isomeric isopelletierine,<sup>4</sup>  $\psi$ -pelletierine,  $\text{C}_9\text{H}_{15}\text{NO}$ , and two isomeric methyl pelletierines,  $\text{C}_9\text{H}_{17}\text{NO}$ , in the root bark of the pomegranate. They are separated by fractional crystallization of the sulphates. The structure of  $\psi$ -pelletierine has been most carefully investigated, and is specially interesting as giving rise to an 8-carbon ring by exhaustive methylation (Part I, p. 186). It forms a methiodide and an oxime, and contains therefore a tertiary nitrogen and a ketone group. It yields on reduction a secondary alcohol, *N*-methyl granatoline, which with hydriodic acid is transformed into the unsaturated *N*-methyl-granatene. The resemblance between the chemical behaviour of

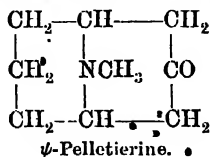
<sup>1</sup> Willstätter and Bode, *Annalen*, 1903, 326, 45.

<sup>2</sup> *Ber.*, 1900, 33, 1160.

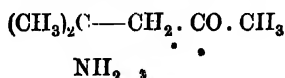
<sup>3</sup> *Ber.*, 1913, 46, 4104.

<sup>4</sup> Hess and Eichel, *Ber.*, 1917, 50, 1192, 1886; 1919, 52, 964.

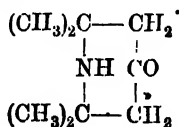
$\psi$ -pelletierine and that of tropinone,  $C_{17}H_{13}NO$ , suggests that the former is a higher homologue. That and its conversion by similar means into cyclo-octadiene by Willstätter<sup>1</sup> leaves no doubt that its structure is to be represented by the formula,



**Euphthalmine and Eucaine.** A very interesting development in synthetic chemistry has arisen out of the knowledge of the structure of atropine and cocaine. The di- and tri-acetonamines are compounds which are obtained by the action of ammonia on acetone, and are represented by the following formulae:

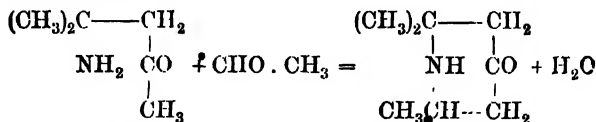


Diacetonamine.



Triacetonamine.

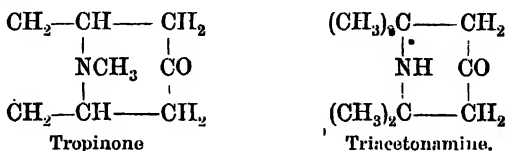
Diacetonamine may be converted into triacetonamine by heating it with acetone. Aldehydes unite in a similar fashion, acetaldehyde forming vinylldiacetonamine.



Diacetonamine.

Vinylldiacetonamine.

The ring complexes formed in this way have a similar structure to tropinone.

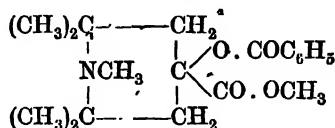


This similarity is not limited to structure, for Harries<sup>2</sup> has shown that the triacetonamines undergo reduction and form what are known

<sup>1</sup> Ber., 1907, 40, 957.

<sup>2</sup> Annalen, 1897, 296, 328; V. Coblenz, J. Soc. Chem. Ind., 1898, 725; 1904, 93.

as *triacetonalcamines* corresponding in structure to tropine, and which, like tropine, form tropeines with acids. Thus the phenylglycolyl ester of *N*-methyl vinylldiacetonalkamine is used as a substitute for atropine under the name of *euphthalmine*, and has a strong mydriatic action. Similarly, Merling has combined the triacetonalamines with hydrogen cyanide, and by hydrolysing the product obtained hydroxy-acids constituted like ecgonine. These substances, when converted into the benzoyl methyl esters, produce local anaesthesia like cocaine, and are known as *eucaïnes*. A variety of these products has been prepared.



Eucaïne A.

Eucaïne A is derived from triacetonalamine, eucaïne B is the benzoyl ester of vinylldiacetonalkamine.

### THE ISOQUINOLINE ALKALOIDS

The alkaloids of this group comprise *harmine* and *harmaline*, the three opium alkaloids, *papaverine*, *narcotine*, *narceïne*, and the two alkaloids, *hydrastine* and *berberine*, which occur in the roots of golden seal (*hydrastis canadensis*) and others of less importance. Berberine, it should be added, is one of the few alkaloids which is distributed among many different orders of plants, such as the common barberry (*berberis vulgaris*), from which it receives its name.

**Harmine and Harmaline.** These alkaloids occur together in seeds of *peganum harmala*, a plant which grows on the steppes of S. Russia and India. They are colourless, crystalline substances which have the molecular formulae :

Harmine,  $\text{C}_{13}\text{H}_{14}\text{ON}_2$ .

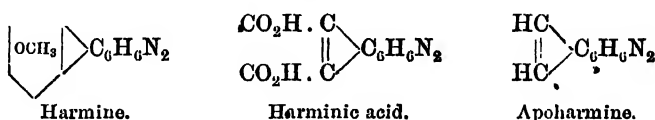
Harmaline,  $\text{C}_{13}\text{H}_{14}\text{ON}_2$ .

<sup>1</sup> The following table, which is taken from Pictet's treatise on the *Vegetable Alkaloids*, gives some idea of the complex nature of opium. The list does not exhaust the number of constituents, which embraces more than twenty different individuals, nor does it include the various other ingredients of the dried sap of the poppy such as fats, resins, gums, sugar, and protein matter.

	Per cent.		Per cent.
Morphine	9.0	Laudanine	0.01
Narcotine	5.0	Lanthopine	0.006
Papaverine	0.8	Protophne	0.003
Thebaine	0.4	Codamine,	0.002
Codeine	0.3	Iritopine	0.0015
Narceïne	0.2	Laudanosino	0.0008
Cryptopine	0.08	Meconine	0.3
Pseudopmorphine	0.02	Meconic acid	4.0
	Lactic acid	1.2	

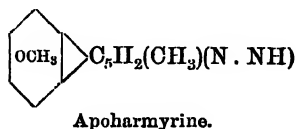
They have been examined by O. Fischer and his co-workers,<sup>1</sup> who have published a series of papers on the subject dating from 1885, and also by W. H. Perkin and Robinson.<sup>2</sup>

Their constitution has been determined from considerations which will now be briefly stated. Harmaline, which contains two hydrogen atoms more than harmine, gives the latter on oxidation, and is therefore, dihydroharmine. They are secondary mono-acid bases, forming salts with one equivalent of acid. Both yield *harminic acid*,  $C_8H_6N_2(CO_2H)_2$ , on oxidation, a substance which forms a phthalein with resorcinol and sulphuric acid, and, on further oxidation, isometoncic acid. When harminic acid is heated it loses successively one and two carboxyl groups and gives *apoharmine*,  $C_8H_8N_2$ . Further, harmaline yields *m*-nitroanisic acid with nitric acid, a reaction in which the basic portion is removed. The relation of harmine to apoharmine may therefore be represented as follows:



Apoharmine is a colourless, crystalline compound which, like the parent substance, is a mono-acid, secondary base.

As harmine condenses with aldehydes giving derivatives of the general formula  $C_{12}H_9ON_2 \cdot CH:OHR$ , it probably contains an  $\alpha$ -methyl group in a pyridine or pyrrole ring (p. 298), so that the structure of the basic constituent of the alkaloids,  $C_6H_6N_2$ , may be further resolved into

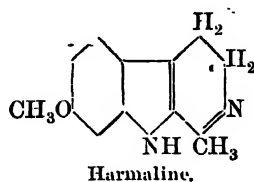
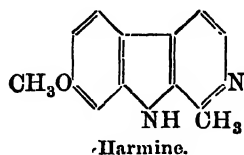


that is, the methyl, methoxy derivative of the parent base,  $C_6H_6N_2$ , termed *apoharmyrene*. The general properties and stability of this group indicate that it has a double cyclic nucleus, which may consist of a condensed 6- and 5-atom ring, of which the nitrogen atoms form part and are present in the same or distributed between the two nuclei. A careful consideration of various alternative formulae

<sup>1</sup> Ber., 1885, 18, 405; 1889, 22, 687; 1897, 30, 2483; 1905, 38, 329; 1912, 45, 1934.

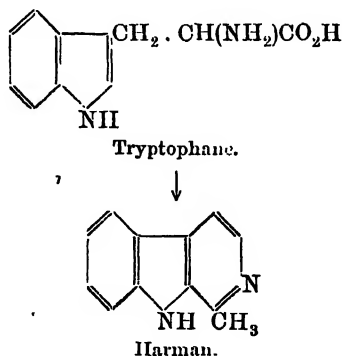
<sup>2</sup> Trans. Chem. Soc., 1912, 101, 1775.

have led Perkin and Robinson to the conclusion that the following are the most probable formulae for harmine and harmaline.<sup>1</sup>



Perkin and Robinson have succeeded in eliminating the methoxy-group from harmine, and the resulting compound is termed *harman*.

Harman was obtained some time ago by Hopkins and Cole<sup>2</sup> by the oxidation of tryptophane, though it is not clear how the third ring structure is formed.

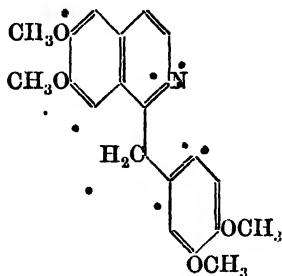


**Papaverine.** The alkaloid was discovered in 1848 by Merck in commercial narcotine, from which it was separated by crystallizing the hydrochloride, the former being less soluble. Merck gave it the formula  $C_{20}H_{21}NO_4$ , which other observers have since confirmed.

<sup>1</sup> *Trans. Chem. Soc.*, 1919, 115, 933.

<sup>2</sup> *J. Physiol. Chem.*, 1903, 29, 451.

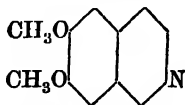
The constitution of papaverine has been studied and completely elucidated by G. Goldschmidt<sup>1</sup> in a series of brilliant researches which appeared in the *Monatsh.* during the years 1883 to 1888. From the results of his investigations, of which a short abstract is given below, he assigned the following formula to the alkaloid:



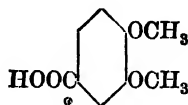
Papaverine.

Papaverine is a tertiary base and optically inactive. It forms no acetyl derivative, and therefore contains no hydroxyl groups. With concentrated hydriodic acid four molecules of methyl iodide are eliminated, and *papaveroline*,  $C_{16}H_{13}NO_4$ , is produced.

On oxidation with permanganate under varying conditions a variety of products are formed, among which may be mentioned *papaveraldine*,  $C_{20}H_{19}NO_5$ , *papaveric acid*,  $C_{16}H_{13}NO_7$ , and simpler compounds derived from these by further oxidation, namely dimethylprotocatechuic acid, metahemipinic acid, dimethoxyisoquinoline carboxylic acid,  $\alpha$ -carbocinchomeric acid, &c. On fusion with alkali, papaveraldine is decomposed into *dimethoxyisoquinoline* and *dimethylprotocatechuic acid*:



Dimethoxyisoquinoline.



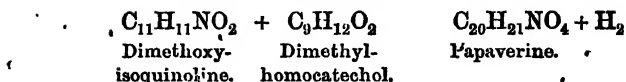
Dimethylprotocatechuic acid.

whilst papaverine on fusion with potash yields dimethylhomocatechol and basic substances.

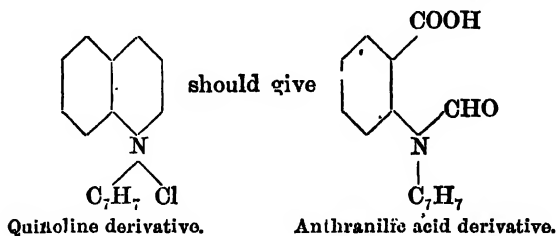
These reactions furnish the key to the structure of the alkaloid. By combining the formula of dimethoxyisoquinoline with that of dimethylhomocatechol, the formula of papaverine is obtained, plus two atoms of hydrogen.

<sup>1</sup> See various papers in *Monatsh.*, 4, 6, 7, 8, 9, 10, 13, 17.

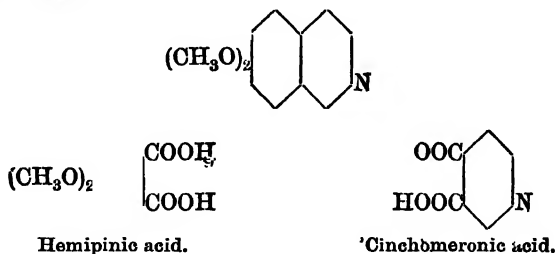




Goldschmidt at first, regarded the isoquinoline compound as a derivative of quinoline until he discovered his error in attempting to ascertain the positions of the two methoxyl groups in dimethoxyisoquinoline by the oxidation of the additive compound with benzylchloride. Instead of the expected derivative of anthranilic acid, he obtained an imide of dimethoxy-phthalic acid (metahemipinic acid).

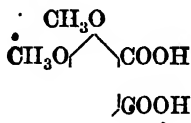


He confirmed this result by oxidising dimethoxyisoquinoline. If the latter is a quinoline derivative it should yield quinolinic acid; if, on the other hand, it is an isoquinoline compound it should form cinchomeronic acid. It was the second reaction which occurred, the compound breaking up in two directions and forming hemipinic and cinchomeronic acid.



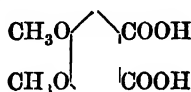
The hemipinic acid formed in this way is so similar to one obtained by Wöhler by the oxidation of narcotine that their identity was at first assumed; but closer examination revealed a slight difference in

properties. Both hemipinic acids readily form anhydrides, and therefore contain the carboxyl groups in the ortho position. Ordinary hemipinic acid, which has been the object of a careful study by Wegscheider, has the formula,



Hemipinic acid.

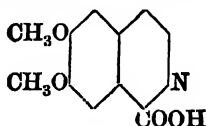
Since the hemipinic acid, which Goldschmiedt calls metahemipinic acid, gives protocatechuic acid on fusion with caustic potash, the only possible structure is:



*m*-Hemipinic acid.

To construct a satisfactory formula for the alkaloid, it is necessary to explain the character of papaverine and the properties of its numerous decomposition products.

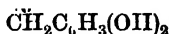
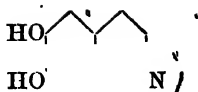
Among the simpler products of oxidation we have still to account for dimethoxy-isoquinoline carboxylic acid and  $\alpha$ -cinchomeronic acid. These are clearly related, since they both are derived from an isoquinoline complex. As the formula for  $\alpha$ -carbocinchomeronic acid is known, that of dimethoxy-isoquinoline carboxylic acid follows;



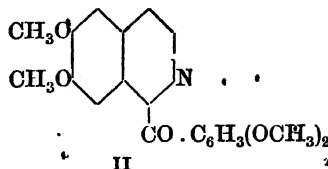
Dimethoxy-isoquinoline carboxylic acid.

for this is the only position which can be assigned to the carboxyl group consistent with the existence of an isoquinoline group, the formation of metahemipinic acid, and the formula of carbocinchomeronic acid.

The formula for papaverine, already referred to, supplies all the necessary demands on the part of the compounds derived from it. Papaveroline, which is produced by eliminating the methoxyl groups, will have the formula I. Papaveraldine, the first product of oxidation, forms a hydrazone, and exhibits the general behaviour of a ketone, and consequently has formula II.



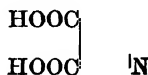
Papaverofline.



II

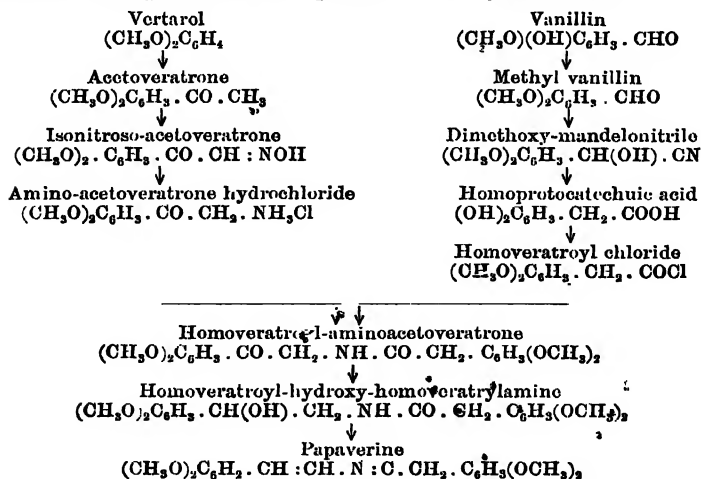
Papaveraldine.

Papaveric acid is a ketonic dibasic acid, which, on fusion with caustic potash, forms protocatechuic acid, and therefore has the formula :



Papaveric acid.

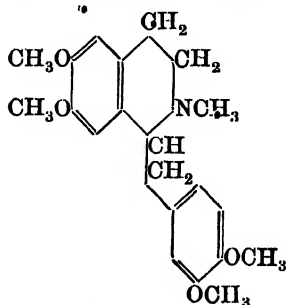
The work of Goldschmiedt on papaverine has prepared the way for the investigation of the other isoquinoline alkaloids which have been attacked by similar methods and with equally successful results, and the correctness of his conclusions has been confirmed by the complete synthesis of papaverine by Pictet and Gams in 1909<sup>1</sup> by the series of steps which are represented briefly as follows :



<sup>1</sup> *Compt. rend.*, 1909, 149, 210. *Ber.*, 1909, 42, 2943.

**Laudanosine** is another opium alkaloid closely related to papaverine, and giving rise to similar degradation products. It has the formula  $C_{21}H_{27}NO_4$ , and therefore contains one carbon atom and six hydrogen atoms more than papaverine. Its constitution was determined by Pictet and his co-workers<sup>1</sup> by the reduction of papaverine methochloride with tin and hydrochloric acid, and subsequently by synthesis carried out by a very similar series of reactions to those described above by condensing homoveratrylamine with homoveratroyl chloride and then reducing the papaverine methochloride to methyl tetrahydropapaverine.

The substance is therefore represented by the following formula:

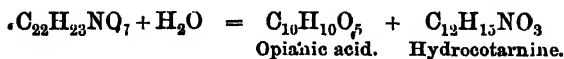


Laudanosine.

The inactive alkaloid was resolved into its active components by crystallization of its quinic acid salts, and the dextrorotatory enantiomorph proved to be identical in every respect with the natural base.

**Narcotine** was isolated from opium in 1817 by Robiquet. The quantity, which varies considerably (0.75–9 per cent.), may be extracted by simply shaking with ether. The molecular formula assigned by Matthiessen and Foster is  $C_{22}H_{23}NO_7$ . It is a mono-acid tertiary base and contains no hydroxyl group, as it reacts neither with acetyl chloride nor acetic anhydride. It contains three methoxyl radicals which may be successively removed. As caustic potash decomposes narcotine at 220°, liberating methylamine, dimethylamine, and trimethylamine, it may be assumed that the nitrogen in the compound is methylated. Narcotine undergoes simple decomposition in three directions. It is hydrolysed by water at 140°, dilute sulphuric, or baryta, and yields *opionic acid* and *hydropotarnine*.

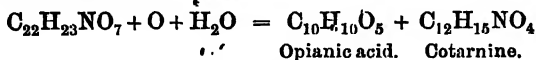
<sup>1</sup> Ber., 1900, 33, 2846; 1909, 42, 1979.



Reducing agents like zinc and hydrochloric acid break it up into *meconine* and hydrocotarnine,

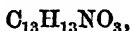


whilst oxidising agents affect the basic part of the molecule and produce opianic acid and *cotarnine*.



We will begin by studying the basic constituent of the molecule.

**Structure of Hydrocotarnine and Cotarnine.** Cotarnine was first obtained by Wöhler in 1844 by oxidising narcotine with manganese dioxide and sulphuric acid, and he gave it the formula

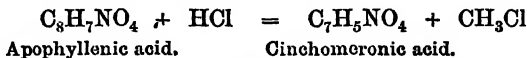


which Matthiessen and Foster afterwards replaced by

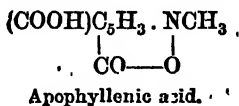


Roser has since shown that the salts of cotarnine contain the basic group,  $C_{12}H_{13}NO_3$ , united with the acid, and that the molecule of water is constitutional. Cotarnine is a secondary base, and forms an oxime with hydroxylamine hydrochloride. When reduced with zinc and hydrochloric acid it is converted into hydrocotarnine.

One of the most interesting derivatives of cotarnine is *apophyllenic acid*, which is obtained by oxidation, and was so called by Wöhler from the resemblance of the crystals to the mineral apophyllite. It is a monobasic acid of the formula  $C_8H_7NO_4 + H_2O$ . According to Vongerichten, when heated with hydrochloric acid to  $250^\circ$ , it loses methyl chloride and forms cinchomeronic acid.

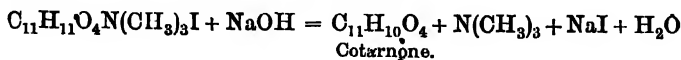


Roser accomplished the reverse synthetic process by boiling cinchomeronic acid with methyl iodide, from which he concluded that apophyllenic acid is the methylbetaine of cinchomeronic acid, leaving undecided which of the two carboxyls takes part in the anhydride formation.

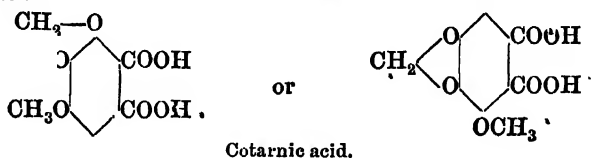


# STRUCTURE OF HYDROCOTARNINE AND COTARNINE 329

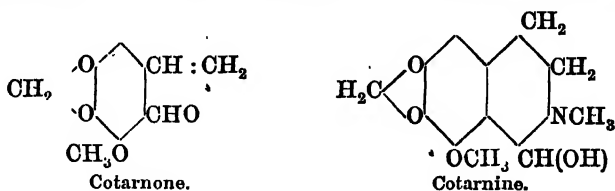
The explanation of the structure of cotarnine is mainly due to the researches of Roser.<sup>1</sup> By exhaustive methylation, a methiodide of methylcotarnine is obtained, indicating thereby the secondary nature of the base. The hydroxide breaks up on distillation, yielding up its nitrogen as trimethylamine and forming *cotarnone*, a nitrogen-free compound with aldehyde properties.



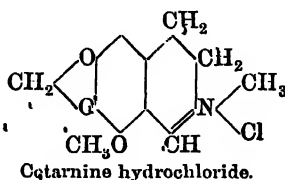
On oxidation with permanganate cotarnone is converted into the dibasic *cotarnic acid*,  $\text{C}_8\text{H}_6\text{O}_2(\text{COOH})_2$ , which Roser, for the reasons given below, regards as having one of the following alternative formulae:



It forms an anhydride, loses one methyl group by Zeisel's method, and, on being heated with hydriodic acid and phosphorus, is converted into gallic acid. Putting these facts together, the above relationships will be best interpreted by the following formulae:

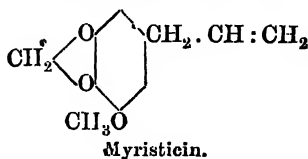


Although cotarnine itself appears not to possess a pyridine nucleus, it is supposed that in its salts ring-formation occurs, and that the hydrochloride of cotarnine is represented by a formula, which is that of a derivative of isoquinoline:

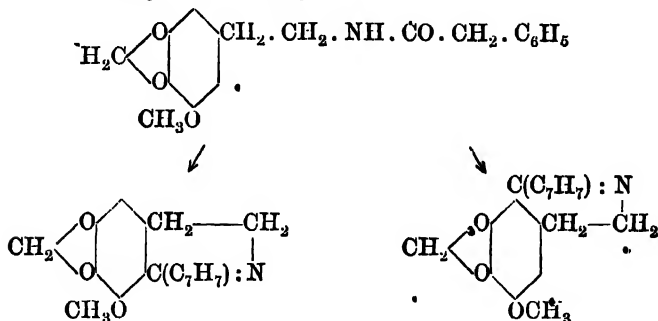


<sup>1</sup> *Annalen*, 1888, 249, 156; 1889, 254, 334.

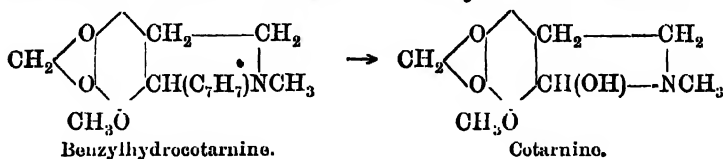
Any doubt which might exist as to the structure of cotarnine has been removed by Salway's synthesis of the base from myristicin.<sup>1</sup>



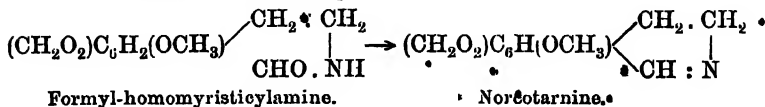
This was converted into the corresponding phenylpropionic acid, propionamide, and ethylamine. The phenacetyl derivation of the base was then treated with phosphoric oxide in xylene solution, when two isomers were formed.



The first isomer was converted into the methochloride and reduced. The product benzylhydrocotarnine on oxidation yielded cotarnine.



The method may be modified by starting from the formyl derivative of homomyristicylamine, which is readily converted into norcotarnine and with methyl iodide into cotarnine methiodide.<sup>2</sup>



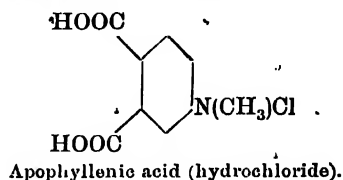
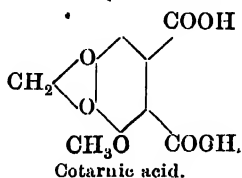
<sup>1</sup> *Trans. Chem. Soc.*, 1910, 97, 1208.

<sup>2</sup> Decker and Beckof, *Anhalen*, 1913, 395, 323.

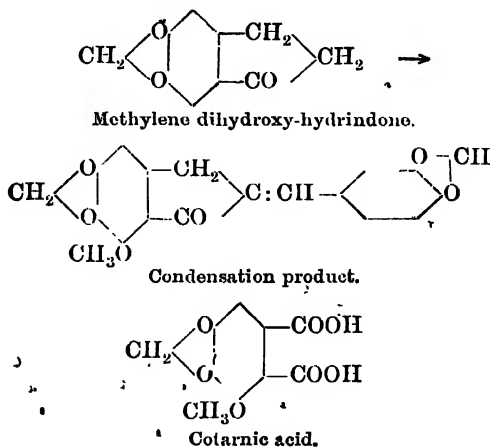
# STRUCTURE OF HYDROCOTARNINE AND COTARNINE 331

According to Dobbie, Lauder, and Tinkler, who have studied its absorption spectrum, cotarnine exhibits tautomerism, passing from the ammonium base, having the structure corresponding to the hydrochloride, to the pseudo-ammonium base, with the structure represented by the above formula, according to whether it is dissolved in a dissociating solvent such as water or a non-dissociating solvent like ether (Part II, p. 77).

The parallelism between the behaviour of isoquinoline and cotarnine on oxidation is very clearly exhibited by the products of oxidation, for whilst the former gives phthalic and cinchomeronic acids, the latter breaks up into cotarnic and apophyllenic acids.

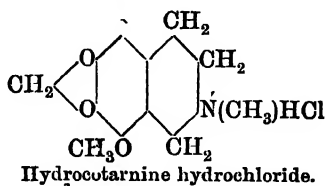


This structure for cotarnic acid has been confirmed by Perkin, Robinson, and Thomas<sup>1</sup> by effecting its synthesis from methylene-dihydroxy-hydrindone. This was nitrated, the nitro group reduced and replaced by hydroxyl, and the latter methylated. The product was condensed with piperonal and oxidised with permanganate, when cotarnic acid was formed.

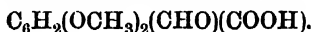




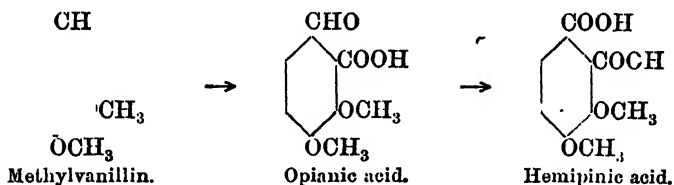
Hydrocotarnine is a reduction product of cotarnine and is also a tertiary base, and these facts are combined in the following formula :



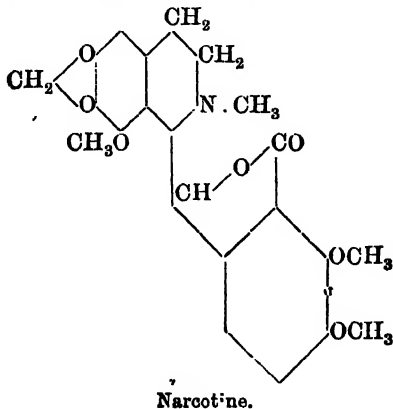
**Opianic Acid.** Opianic acid is a monobasic acid having the properties of an aldehyde and containing two methoxyl groups. Its formula may therefore be represented by



The relative positions of these groups has been ascertained as follows : on distillation with soda-lime it yields methylvanillin, and on oxidation it is converted into hemipinic acid (p. 336).

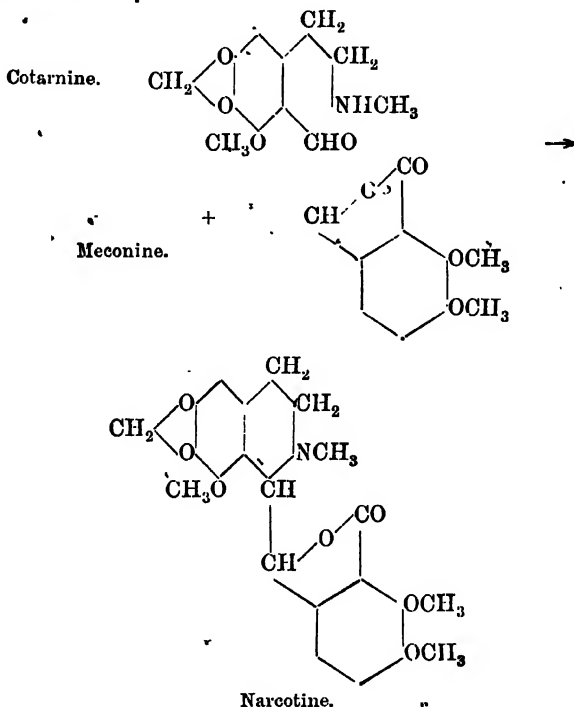


Finally, *meconine* is the lactone of the alcohol produced by the reduction of the aldehyde group in opianic acid.



# STRUCTURE OF HYDROCOTARNINE AND COTARNINE 333

The synthesis of inactive narcotine, which is identical with the naturally occurring *gnoscopine*, has been effected by Perkin and Robinson<sup>1</sup> by warming a mixture of meconine and cotarnine in alcoholic solution.

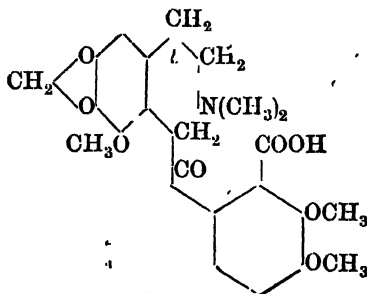


**Narceine.** The relationship of narcotine to narceine has been established by Roser's synthesis of the latter from narcotine. When the methiodide of narcotine is heated with caustic alkali it yields narceine.



Its other properties are in harmony with the following structural formula:

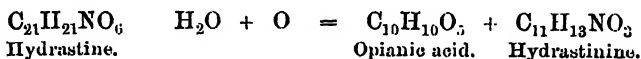
<sup>1</sup> *Trans. Chem. Soc.*, 1911, 99, 775.



Narceine.

We shall close our account of the isoquinoline alkaloids with a short reference to hydrastine and berberine.

**Hydrastine.** The root of the golden seal (*Hydrastis canadensis*), a plant belonging to the Ranunculaceae and indigenous to N. America, contains about 1.5 per cent. of hydrastine and 4 per cent. of berberine. Hydrastine was first observed by Durand in 1851, and has since been studied by numerous investigators. Our knowledge of its structure is mainly due to the work of E. Schmidt<sup>1</sup> and M. Freund<sup>2</sup> during the last two decades. It is closely related to narcotine. Like narcotine, it breaks up by acid oxidation into *opianic acid* and *hydrastinine*.



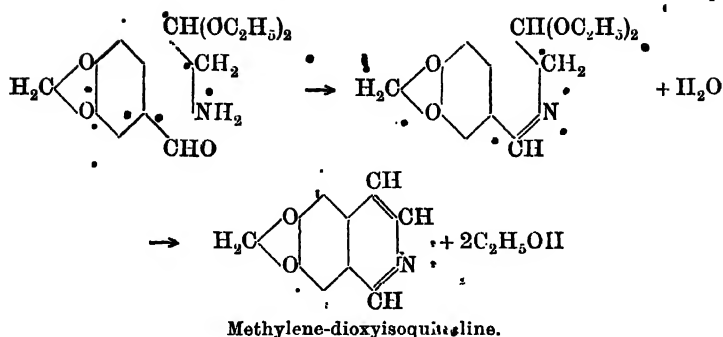
It contains two methoxyl groups, but neither aldehyde nor ketone group nor ethylene linkage.

Hydrastinine only differs from cotarnine by the group  $\text{CH}_2\text{O}$ , which suggests one methoxyl group less in hydrastinine, and leads to the general conclusion that narcotine itself is a methoxy-hydrastine. This view has been confirmed. Hydrastinine on reduction with zinc and hydrochloric acid, sodium amalgam, or by electrolysis, is converted into *hydrohydrastinine*,  $\text{C}_{11}\text{H}_{13}\text{NO}_2$ , a compound which has been synthesised by Fritsch<sup>3</sup> as follows: piperonal is condensed with amino-acetal, and the product is then treated with sulphuric acid, by which alcohol is removed.

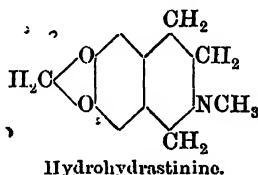
<sup>1</sup> *Archiv d. Pharm.*, 1884, 224, 974; 226, 239; 1885, 228, 49, 241, 596; 231, 541; 1886, 232, 136.

<sup>2</sup> A summary is contained in *Annalen*, 1892, 271, 311.

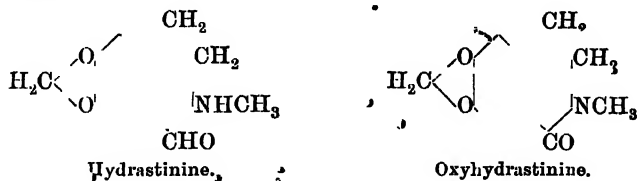
<sup>3</sup> *Annalen*, 1895, 286, 1.



When the methiodide of the last compound is reduced with tin and hydrochloric acid, it is converted into hydrohydrastinine. According to Freund, hydrohydrastinine yields hydrastinine on oxidation with potassium dichromate and sulphuric acid.



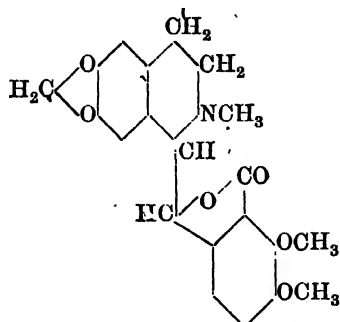
The relation of hydrohydrastinine to hydrastinine follows from the general properties of the latter. It contains an aldehyde group like cotarnine, and, like cotarnine, forms salts with the elimination of a molecule of water. On oxidation it is converted successively into oxyhydrastinine  $C_{11}H_{11}NO_3$ , hydrastinic acid  $C_{11}H_9NO_6$ , and finally apophyllenic acid. There are a variety of other products known, but sufficient has been stated to afford a basis for a satisfactory structural formula. The relation of hydrastinine to oxyhydrastinine is represented as follows:



Finally, norhydrastinine and hydrastinine have been synthesised by a method similar to that described under cotarnine (p. 330).<sup>1</sup>

<sup>1</sup> Decker and Becker, *Annalen*, 1913, 395, 328; see also Rosenmund, *Abstr.*, 1919, i, 280.

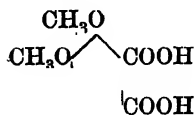
Hydrastine has the formula :



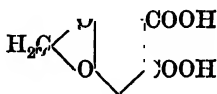
Hydrastine.

**Berberine** was discovered in 1826 in prickly ash (*xanthoxylum claraherculis*) by Chevalier and Pelletan, who named it *xanthopicroite*. When Büchner, in 1835, found it in barberry root the name was changed to berberine. Since then it has been observed in golden seal and many other plants, the largest amount (8-9 per cent.) being found in *coptis*. The composition of berberine,  $C_{20}H_{17}NO_4$ , as well as its constitution, have been worked out very completely by W. H. Perkin, jun.<sup>1</sup> The following are the principal facts upon which its structure rests. Berberine is a tertiary base, and forms salts which have a yellow colour. It contains neither aldehyde, ketone, nor hydroxyl group. Of the two methyl groups, which are removed by Zeisel's method, neither is attached to nitrogen, and they are consequently present as methoxyl.

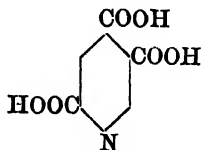
The most important insight into the structure of berberine is afforded by its products of oxidation. By the action of permanganate in alkaline solution, Schmidt<sup>2</sup> obtained *hemipinic* and *hydrastic acids*, both of which had been previously prepared from hydrastine; whilst Weidel,<sup>3</sup> by using concentrated nitric acid, obtained *berberonic acid*, or  $\beta$ - $\gamma$ - $\alpha'$ -pyridine tricarboxylic acid.



Hemipinic acid.



Hydrastic acid.



Berberonic acid.

<sup>1</sup> *Trans. Chem. Soc.*, 1889, 55, 63; 1890, 57, 992; 1910, 97, 305.

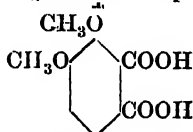
<sup>2</sup> *Arch. d. Pharm.*, 228, 596.

<sup>3</sup> *Ber.*, 1879, 12, 410.

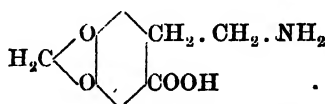
A renewed study of the action of permanganate on berberine by Perkin, jun., led to the isolation of the following series of oxidation products:

Oxyberberine	$C_{20}H_{17}NO_5$	Anhydroberberilic acid	$C_{20}H_{17}NO_8$
Dioxyberberine	$C_{20}H_{17}NO_6$	Berberilic acid	$C_{20}H_{19}NO_9$
Berberal	$C_{20}H_{17}NO_7$	Berberilic acid	$C_{20}H_{15}NO_8$

from which oxyhydrastinine was ultimately prepared and a common bond established with narcotine and hydrastine. The most interesting of the above series are *berberilic acid* and *berberal*. Berberilic acid, which is a dibasic acid, breaks up, on boiling with dilute sulphuric acid into hemipinic acid and amino-ethyl piperonylic acid.

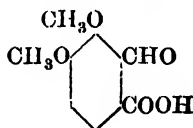
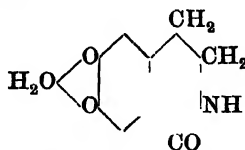


Hemipinic acid.



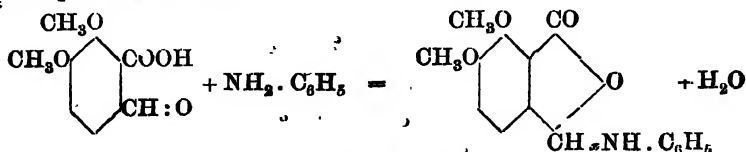
Amino-ethyl piperonylic acid.

In the same way berberal, when treated with alcoholic potash, is resolved into amino-ethyl piperonylic anhydride,  $C_{10}H_9NO_3$ , and  $\psi$ -opianic acid,  $C_{10}H_{10}O_5$ , the two constituents being again combined to form berberal by heating them together to  $180^\circ$ . Amino-ethyl piperonylic anhydride is regarded by Perkin as noroxyhydrastinine, since it can be converted into oxyhydrastinine.

 $\psi$ -Opianic acid.

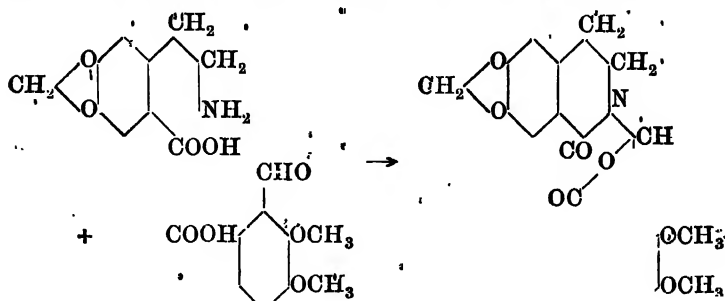
Noroxyhydrastinine.

Now it has been shown that when opianic acid (which only differs from  $\psi$ -opianic acid by inverting the positions of aldehyde and carboxyl groups) reacts with basic substances it is the carbon of the aldehyde group which attaches itself to the nitrogen of the base.<sup>1</sup> Thus, Liebermann has found that aniline and opianic acid form a compound as follows:



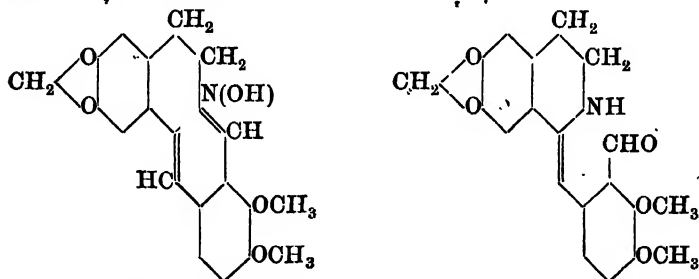
<sup>1</sup> Perkin and Robinson, *Trans. Chem. Soc.*, 1910, 97, 320.

As opianic and  $\psi$ -opianic acid closely resemble one another in chemical behaviour, the union of the latter with amino-ethyl piperonyl carboxyl anhydride will be represented as follows:



Berberal.

The latter will therefore represent the formula for berberal, and berberine will then be



Berberine (ammonium form).

Berberinal (aldehyde form).

Berberine in presence of alkali undergoes an intermolecular change whereby it assumes the functions of an aldehyde and is termed *berberinal*. In this form it closely resembles hydrastine in structure, and it is not surprising to find that hydrastinine bases may be obtained from berberine derivatives,<sup>1</sup> and that berberine may be converted into hydrastinine.<sup>2</sup>

The correctness of this formula has been confirmed by the synthesis of berberine and oxyberberine by Pictet and Gams.<sup>3</sup> Piperonal condenses with nitromethane and the product on oxidation and subsequent reduction yields successively the aldoxime and amine.

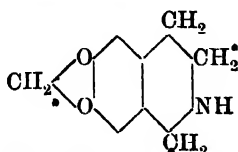


<sup>1</sup> Freund, *Abstr.*, 1912, i. 383.

<sup>2</sup> Merck, *Abstr.*, 1913, i. 1095.

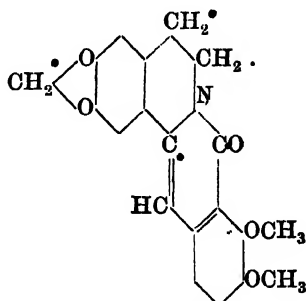
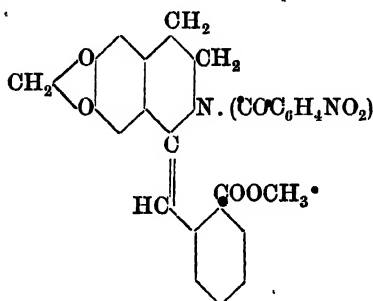
<sup>3</sup> *Compt. rend.*, 1911, 152, 1102; 153, 386.

On treating the amine with formaldehyde in presence of hydrochloric acid norhydrodrastinine is formed.



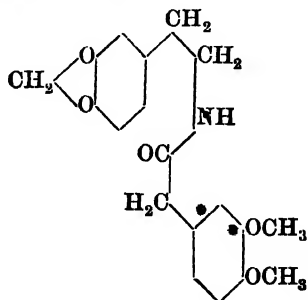
Norhydrodrastinine.

The nitrobenzoyl derivative combines with the methyl ester of opianic acid, and the product on treatment with alcoholic potash gives oxyberberine.



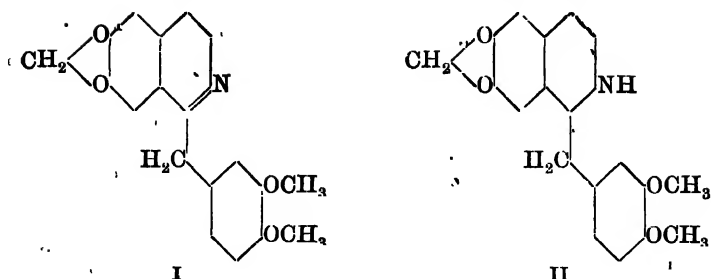
Oxyberberine.

Berberine itself was obtained by combining homoveratroyl chloride with homopiperonylamine thus:

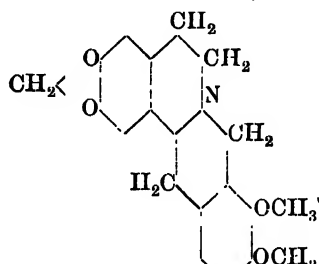


When heated with phosphorus pentoxide a molecule of water is eliminated, and the product (I) on reduction yields veratroyl-norhydrodrastinine (II).





By the action of methylal in presence of hydrochloric acid a  $\text{CH}_2$  group is introduced and unites the isoquinoline with the veratroyl nucleus, thus yielding tetrahydroberberine, which gives berberine on oxidation.



Tetrahydroberberine.

Tetrahydroberberine is the inactive form of another laevo-rotatory alkaloid found in golden seal (*hydrastis canadensis*) and known as *canadine*. By fractional crystallization of the *d*-bromocamphor-sulphonate Gadamer<sup>1</sup> resolved the inactive compound into its components and obtained the laevo form, which was identical with *conadine*.

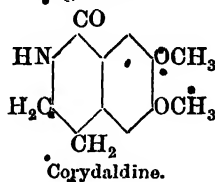
**Corydaline** is the chief constituent among the alkaloids occurring in *corydalis cava*. It is a colourless crystalline substance of the formula  $\text{C}_{22}\text{H}_{27}\text{O}_4\text{N}$ , and is dextro-rotatory. For its structure we are mainly indebted to the work of Dobbie and Lauder, who have summarized their results in a paper published in 1902,<sup>2</sup> of which the following is a brief account:

By the action of mild oxidising agents, such as dilute nitric acid, four atoms of hydrogen are removed and an intensely yellow base, *dehydrocorydaline*,  $\text{C}_{22}\text{H}_{23}\text{O}_4\text{N}$ , is produced, which on reduction is converted into the original, but inactive, compound. In this and in

<sup>1</sup> *Arch. Pharm.*, 1894, 232, 186.

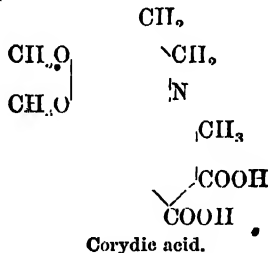
<sup>2</sup> *Trans. Chem. Soc.*, 1902, 82, 145.

many other respects dehydrocorydaline resembles berberine, which is also yellow, and on reduction forms a colourless tetra-hydro derivative. It contains four methoxy groups, and on oxidation with a hot solution of permanganate it gives hemipinic and *m*-hemipinic acids. Oxidised at the ordinary temperature it yields *corydaldine*, which has been shown to possess the following structure:<sup>1</sup>

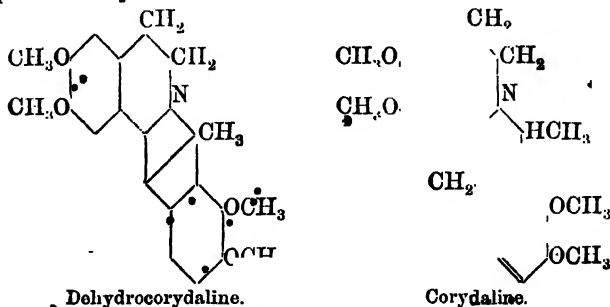


It therefore contains an isoquinoline nucleus. If nitric acid is used as oxidising agent in place of permanganate, a dibasic acid, corydic acid,  $C_{18}H_{17}O_6N + \frac{1}{2}H_2O$ , is formed, which on further oxidation yields *corydile acid*,  $C_{17}H_{15}O_8N$ , which is tribasic, and which in turn is slowly resolved into methyl pyridine tricarboxylic acid and *m*-hemipinic acid.

Putting these facts together the following structure is assigned to corydic acid:

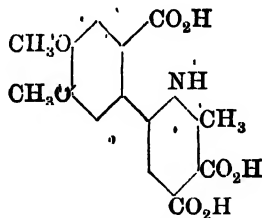


From this it follows that dehydrocorydaline and corydaline will be represented by the formulae:



<sup>1</sup> *Trans. Chem. Soc.*, 1899, 75, 670

a relation, which closely resembles that of berberine and tetrahydroberberine. The formulae account, moreover, for the formation of hemipinic and *m*-hemipinic acids from rings I and IV and methylpyridine tricarboxylic acid from ring II. Corydaldine would contain rings III and IV. Corydic acid would result from the destruction of ring I, and corydilie acid from corydic acid by the oxidation of ring III

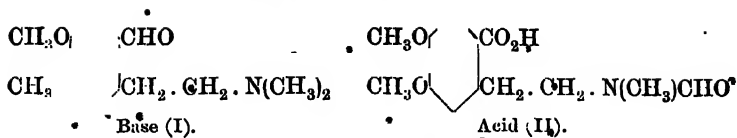


Corydilie acid.

**Cryptopine and Protopine.** These two alkaloids occur in opium, and cryptopine was discovered in 1867 by J. Smiles, in the filtrate from which the morphine and thebaine had been separated. It was first analysed by Hesse, who gave it the formula  $C_{21}H_{23}O_6N$ , which has since been confirmed. Our knowledge of its structure is due in the first place to D. R. Brown and W. H. Perkin, jun., who showed that it contains two methoxy-groups and, on oxidation, yields *m*-hemipinic acid (p. 325). Since then the alkaloid has been the subject of numerous investigations, but it is only within the last year that the problem of its constitution has been thoroughly elucidated by the work of W. H. Perkin, jun.,<sup>1</sup> in a paper from which this brief account is abstracted.

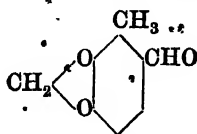
The fact that cryptopine has a molecular formula resembling that of hydrastine,  $C_{21}H_{21}O_6N$ , and berberine,  $C_{20}H_{19}O_6N$ , would indicate a relationship between them, and this conclusion is supported by certain reactions which are shared by the other two alkaloids. Cryptopine undergoes the following changes: It combines with methyl sulphate and forms  $C_{21}H_{23}O_6N_1(CH_3)_2SO_4$ , which on reduction yields *tetrahydro-methyl cryptopine*,  $C_{22}H_{29}O_6N$ . The latter reacts with acetyl chloride, forming the anhydro-derivative  $C_{22}H_{27}O_4N$ , which on oxidation yields a syrupy base,  $C_{15}H_{19}O_3N$ , an aldehyde, and an acid. The base (I) has been identified as possessing the formula:

<sup>1</sup>Trans. Chem. Soc., 1916, 109, 815.

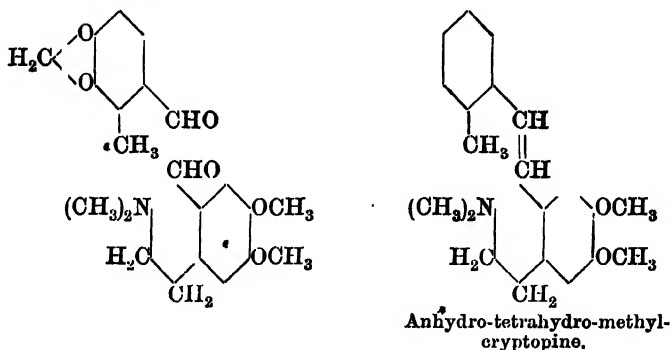


whilst the acid is represented by formula II.

The aldehyde contains no methoxy groups, but a methylene group, and resembles piperonal in smell and certain chemical properties. From its formula, which is that of a methyl piperonal, and its oxidation product it has been shown to have the formula:



By joining together the base with the aldehyde, on the assumption that on oxidation the junction attached is an ethylene linkage, the following structure is obtained:

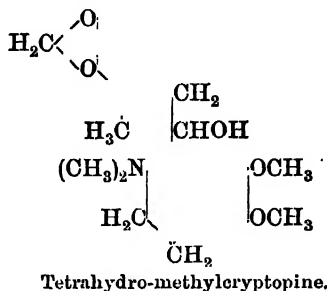
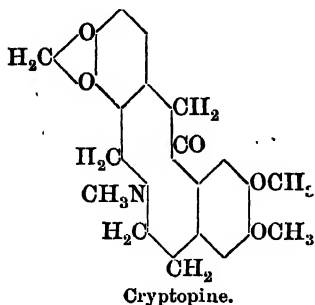


The tetrahydro-compound will probably possess the group formed by adding the elements of water to the middle ethylene linkage, which, of the two alternative arrangements, is probably represented by

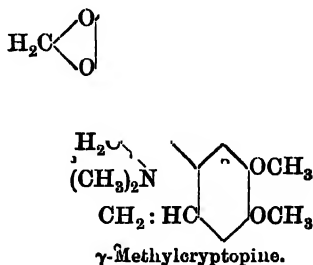
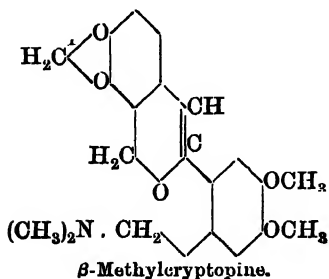


When cryptopine is reduced with a large excess of sodium amalgam in dilute sulphuric acid, it yields a dihydro-derivative  $\text{C}_{21}\text{H}_{25}\text{O}_5\text{N}$ .

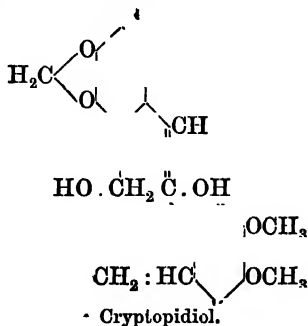
This is explained by the reduction of the original ketone group to the secondary alcohol group of the middle linkage. The addition of the second molecule of hydrogen to form the tetrahydro-compound is determined by the cleavage of the methylene linkage of the piperonal nucleus to form a methyl group. The formulae of cryptopine and tetrahydro-methylcryptopine will be as follows:



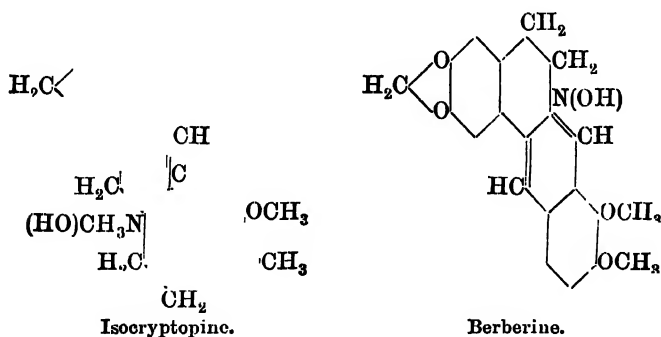
Four oxygen atoms of the molecule are readily accounted for (as methylene oxide and methoxy-groups) whilst the nature of the fifth is not so readily determined, seeing that it exhibits unusually inactive properties. It neither forms an acetyl derivative nor gives the usual ketone reactions. This would point to an ether (C—O—C) grouping had it not been for the inability of such an arrangement to explain the formation and properties of the *methylcryptopines*. These substances are obtained, one ( $\alpha$ ) from the methochloride and two from the methosulphate by the action of alkalis, and one named  $\alpha$ -,  $\beta$ -, and  $\gamma$ -methylcryptopines;  $\alpha$ - being polymorphic and  $\gamma$ - isomeric, with  $\beta$ -methylcryptopine. Unlike the  $\beta$ - modification, the  $\gamma$ -isomer gives an oxime and semicarbazone, as well as an acetyl derivative. The methosulphate of both compounds, when boiled with alcoholic potash, gives *cryptopidiol*, and trimethylamine is eliminated. These reactions are interpreted by means of the following formulae:



whilst cryptopidiol has the constitution :



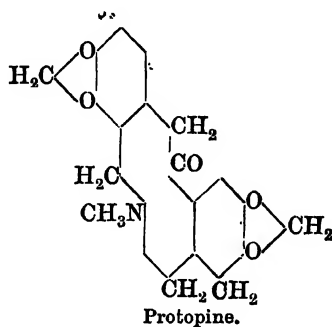
One further reference must suffice to illustrate the innumerable changes which this mobile molecule can assume, and one which at the same time exhibits its relation to berberine (see below). Isocryptopine chloride is most readily obtained by the action of phosphorus oxychloride on cryptopine from which the base may be separated. In this reaction it is assumed that cryptopine passes into the enolic form and undergoes a similar tautomeric change to that of cotarnine (p. 331).



*Protopine*,  $\text{C}_{20}\text{H}_{19}\text{O}_5\text{N}$ , although one of the most widely distributed of the alkaloids, being found in most species of poppy (*Papaveraceae*), in the roots of the greater celandine (*Ciclidonium majus*), in certain species of corydalis (*Corydalis cava*), &c., it usually occurs in very small quantity. It was found by Hesse<sup>1</sup> to accompany cryptopine in opium; but the best source is probably the Lyre flower (*Dicentra*

<sup>1</sup> *Annalen*, Suppl., 1872, 8, 261.

*specabilis*), in which it is present to the extent of 0.7-1.0 per cent. It exhibits in all its properties a striking resemblance to cryptopine, from which it differs in possessing no methoxy-groups. The formula,  $C_{20}H_{19}O_5N$ , contains  $CH_4$  less than cryptopine,  $C_{21}H_{23}O_5N$ , and Gadamer and Danckwóth have consequently suggested that the two methoxy groups in cryptopine are replaced by the methylene oxide group. If this is assumed (and the view is accepted by Perkin as the most probable interpretation) the formula for protopine will be:



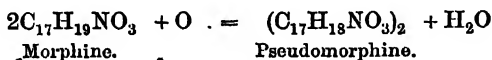
### THE MORPHINE ALKALOIDS

This group includes at least four important alkaloids found in opium, namely, *morphine*, *codeine*, *pseudomorphine*, and *thebaine*. They are distinguished from the more numerous class of opium alkaloids to which papaverine and narcotine belong, by their poisonous character. In spite of the enormous mass of material which has resulted from the study of these alkaloids, we are still ignorant of their structure. The following pages contain a very general and incomplete summary of the results.

**Morphine and Codeine.** The structural relationships between morphine and codeine may at once be made clear. The formula for morphine,  $C_{17}H_{19}NO_3$ , and codeine,  $C_{18}H_{21}NO_3$ , indicates a difference

of a methyl group, and the conversion of the one into the other by Grimaux in 1881 by the action of methyl iodide and caustic potash, and later by that of diazomethane on morphine, leaves no doubt about the correctness of this view.

Morphine, as already stated (p. 264), was the first alkaloid to be isolated. Although the average amount in opium is given in the table (p. 320) as 9 per cent., it varies considerably, and may rise to 20 per cent. or more in some specimens, or fall to 3 per cent. in others. Morphine is a tertiary base and at the same time a monohydric phenol, for it dissolves in caustic alkalis and forms salts with one atom of metal, from which it is again precipitated by carbon dioxide. On the other hand, it forms diacyl derivatives, and therefore contains two hydroxyls, one of which is probably alcoholic in character. The third oxygen is indifferent and is probably present as an anhydride or ether group. Morphine is very oxidisable, reducing certain metallic salts and separating iodine from iodic acid. The product of these and other weak oxidising agents is a non-poisonous compound known as *pseudomorphine*, which is also present in opium. Its structure is still unknown.



The action of dehydrating agents is either to produce condensation of two or more molecules and form tri- and tetra-morphine or, if hydrochloric acid is used, to eliminate water with the production of a substance known as *apomorphine*,  $\text{C}_{17}\text{H}_{17}\text{NO}_2$ , in which two phenolic hydroxyls are present.

Our knowledge of the structure of morphine and codeine from this point centres round the recent investigations of Vongerichten,<sup>1</sup> of Knorr and of Pschorr.<sup>2</sup>

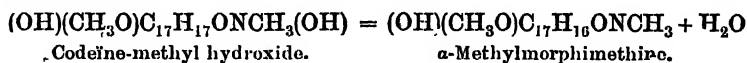
By the distillation of morphine over zinc dust, Vongerichten and Schrötter obtained *phenanthrene* together with a series of bases—ammonia, trimethylamine, pyrrole, pyridine, and a substance, morphidine, since recognized as a mixture of two bases. The appearance of phenanthrene is sufficiently interesting, but in consequence of the high temperature used in the reaction, no proof is afforded of its

<sup>1</sup> See various papers in the *Berichte* from 1896 onwards.

<sup>2</sup> See various papers in the *Berichte*, 1889, 1894, 1897, 1898, 1899, 1903, and *Annalen*, 1898, 301, 1; 1899, 307, 171.



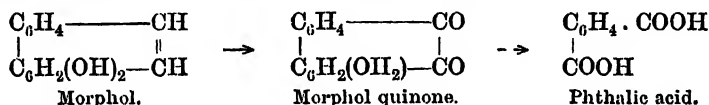
existence in the alkaloid itself. Further confirmation was necessary. Vongerichten and Schrötter then submitted codeine to the process of exhaustive methylation. On distilling codeine-methyl hydroxide a new base is formed, to which the name *α-methylmorphimethine* has been given.



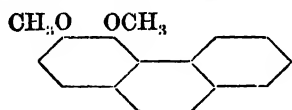
This new compound still contains hydroxyl, and when heated with hydrochloric acid or acetic anhydride is resolved into methyl-dihydroxyphenanthrene and dimethylamino-ethanol.



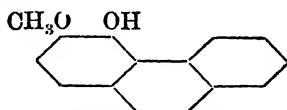
The structure of methyl-dihydroxyphenanthrene was determined by its resolution into dihydroxyphenanthrene or *morphol* and into phenanthrene, and since *morphol* is converted successively by oxidation into the corresponding quinone and into phthalic acid, both hydroxyls must be present in the same ring.



The structure of dimethyl- and monomethyl-morphol has since been confirmed by Pschorr and Sumuleanu and by Pschorr and Vogtherr,<sup>1</sup> who obtained them synthetically.

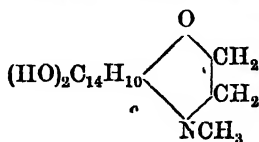


Dimethyl-morphol  
(3,4-Dimethoxyphenanthrene).



Methyl-morphol  
(3-Methoxy-4-hydroxyphenanthrene).

By combining the results of the various reactions carried out in the manner described, Knorr originally suggested a formula for morphine in which the methylamino-ethanol group was attached to the phenanthrene nucleus as an oxazine or *morpholine* group thus:

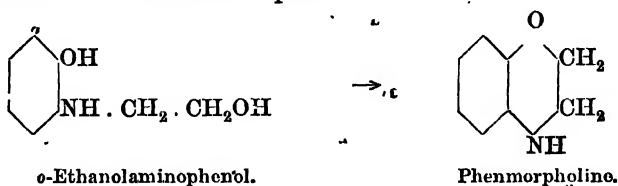


<sup>1</sup> Ber., 1900, 33, 1810; 1902, 35, 4412.

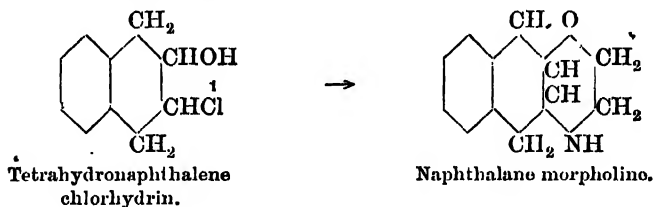
but this view is now abandoned, and the ethanol group is at present regarded by Knorr<sup>1</sup> as a secondary product derived from an original vinyl group.

The reasons for this are based upon the different behaviour of the artificial morpholine derivatives.

A variety of these compounds have been prepared synthetically by Knorr. Such, for example, is *phenmorpholine*, obtained by internal condensation of *o*-ethanolaminophenol.



Another interesting synthetic product is the base *naphthalene morpholine*,<sup>2</sup> which is obtained by condensing tetrahydronaphthalene chlorhydrin with ethanolamine.



Although the *N*-methyl derivative of this compound closely resembles morphine, both in its physiological action and in the character of its disintegration products, yet the dimethylamino-ethanol derivatives of dihydronaphthalene differ greatly in stability from methylmorphimethine, and they cannot therefore be similarly constituted. The same view has been arrived at on other grounds by Freund<sup>3</sup> from the study of the closely related alkaloid, thebaine.

This connection between codeine, morphine, and thebaine has been arrived at in the following way: *codeinone*, which is obtained by the oxidation of codeine, is a ketone and yields, when heated with dilute hydrochloric acid, *thebenine*, and with strong hydrochloric acid, *morphothebaine*. Now, as both these compounds are obtained in the same manner from thebaine, the two alkaloids must be nearly related.<sup>4</sup> Moreover, codeinone is decomposed by acetic anhydride into methylamino-ethanol and 3-methoxy-4,6-dihydroxyphenanthrene,

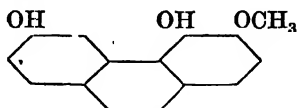
<sup>1</sup> Ber., 1905, 38, 8143.

<sup>3</sup> Ber., 1905, 38, 3234.

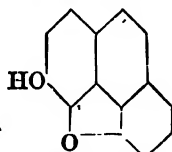
<sup>2</sup> Annalen, 1899, 307, 171.

<sup>4</sup> Ach and Knorr, Ber., 1903, 36, 3067.

which is related to *thebaol* (p. 351). Thus, codeine is a derivative of 8.4.6-trihydroxyphenanthrene.

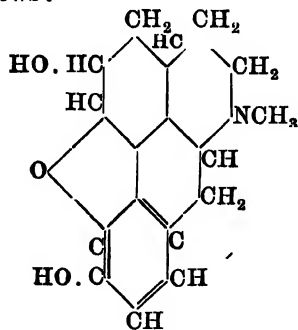


Pschorr<sup>1</sup> regards the nitrogen atom in the three alkaloids as forming part of a pyridine ring and bases his view on the persistence of the 'indifferent' oxygen atom when the nitrogen complex is entirely detached. Thus, Vongerichten obtained from the stereoisomeric or  $\beta$ -methylmorphimethine *morphenol*, which probably has the formula:

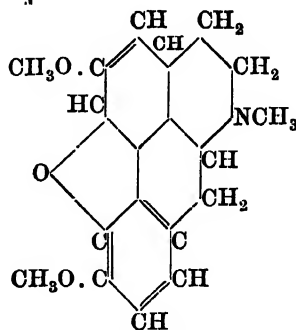


Morphenol.

and Pschorr formulates the structure of morphine and thebaine as follows:



Morphine.



Thebaine.

**Thebaine** was discovered in opium by Thiboumery in 1835. It was investigated by Pelletier, and its composition ( $C_{19}H_{21}NO_3$ ) was correctly determined by Anderson. Our knowledge of its structure, as far as it is known, is mainly the result of the careful and systematic study which Martin Freund<sup>2</sup> has devoted to the subject since 1894.

The molecular formula of the three alkaloids, morphine, codeine, and thebaine, would in itself suggest a connection between them.

<sup>1</sup> The present position of the morphine problem is discussed by Pschorr and Einbeck, *Ber.*, 1907, 40, 1980, and by Knorr and Hörlein, *Ber.*, 1907, 40, 2042.

<sup>2</sup> *Ber.*, 1895, 28, 941; 1897, 30, 1857; 1899, 32, 168.



Such a relationship has already been shown to exist. Thebaïne is a tertiary base; it contains two hydrogen atoms less than morphine no hydroxyl, but two methoxyl groups. As, in addition, it yields tetramethylethylene diamine,



on exhaustive methylation, it contains a *N*-methyl group, and accordingly its formula may be written:

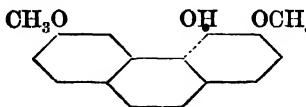


Thebaïne undergoes a similar decomposition to the other two alkaloids; for on boiling with acetic anhydride it is resolved into the acetyl derivatives of the nitrogen-free *thebaol* and of *methylamino-ethanol*, the latter furnishing a further proof of the presence of the methylamine radical.



Thebaïne methyl iodide in presence of silver acetate undergoes a similar change, but in this case, in addition to thebaol acetate the acetyl derivative of dimethylamino-ethanol is formed.

Thebaol was shown by Freund, using similar methods to those, already described, to be a dimethoxy-hydroxyphenanthrene. It yields a quinone on oxidation resembling phenanthraquinone and possessing the properties of an ortho diketone, and on further oxidation passes into *o*-methoxyphthalic acid. The synthesis of thebaol-quinone by Pschorr and Seydel<sup>1</sup> from 2-nitro-isovanillin has definitely established the structure of thebaol as 4-hydroxy-3:6-dimethoxyphenanthrene.



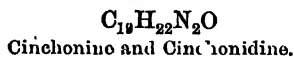
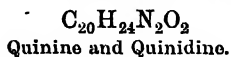
It is clear from the foregoing that the constitution of thebaïne is intimately related to that of morphine and codeïne, and that the same key which would serve to unlock one structure would fit the other two. At present, however, the key is missing, and the structural formulae which have been assigned must be regarded as provisional.

<sup>1</sup> Ber., 1902, 35, 4400.

## THE QUINOLINE ALKALOIDS

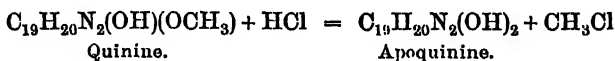
**Quinine and Cinchonine.** Among the numerous alkaloids which are found in cinchona bark, estimated at more than twenty, quinine, quinidine, cinchonine, and cinchonidine are the most plentiful, the most important, and the most carefully investigated. As it has been also shown that they are similarly constituted, there are certain advantages in discussing them together.

These alkaloids were discovered in 1820 by Pelletier and Caventou, and the molecular formulae of the anhydrous bases were found to be:



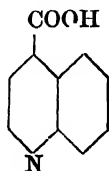
The two pairs are probably stereoisomeric, so that we shall consider only quinine and cinchonine.

Both alkaloids are bi-tertiary bases, that is to say, the two nitrogen atoms are present as tertiary groups. Of the two oxygen atoms of quinine one is present as hydroxyl, the other as methoxyl. By heating quinine with strong hydrochloric acid the methyl group is eliminated as methyl chloride and the product is known as apo-quinine.

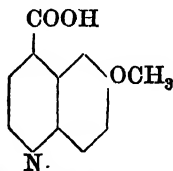


The oxygen atom of cinchonine represents a hydroxyl group.

**Oxidation of Quinine and Cinchonine.** The products obtained by oxidising the two alkaloids has shown that each alkaloid is sharply divisible into two parts. With energetic oxidising agents cinchonine yields cinchoninic acid, whilst quinine yields quininic acid. The structure of both acids is known and represented by the following formulae:

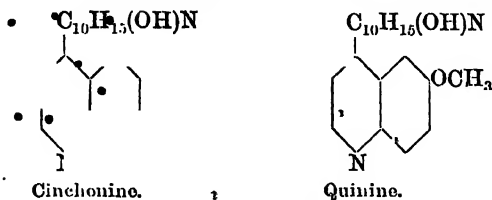


Cinchoninic acid.



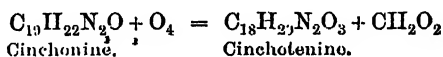
Quininic acid.

It will be seen that the two acids stand in the same relation as the alkaloids from which they are derived, and thus 'the second-half', as it is termed, is probably identical in both.

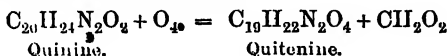


The further investigation of their structure, which up to this point was clearly explained by Skraup, has offered unexpected difficulties, and the constitution of 'the second-half' is not yet finally and definitely established.

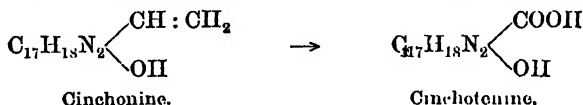
Among the mass of materials which have accumulated on the subject, those only have been selected which appear to have a direct bearing on the problem under discussion. If cinchonine is oxidised with permanganate, formic acid is split off and a new base *cinchotenine* is formed.



Similarly quinine yields *quitenine*.

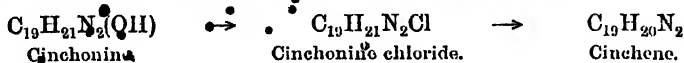


Cinchotenine still contains the original hydroxyl group of cinchonine, and in addition a carboxyl group, since it yields an ester; but whilst cinchonine forms an additive compound with hydrogen iodide, cinchotenine has lost this power. Thus, in all probability, the change depends on the oxidation of an unsaturated side-chain.



The same kind of difference is exhibited between quinine and quitenine.

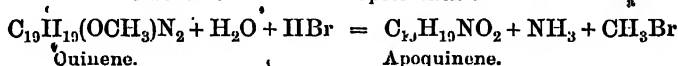
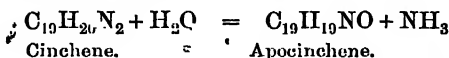
Koenigs<sup>1</sup> found that when cinchonine is acted upon with a mixture of phosphorus pentachloride and oxychloride the hydroxyl group is replaced by chlorine and forms *cinchonine chloride*. Alcoholic potash removes a molecule of hydrogen chloride, and a new oxygen-free base is formed, which was named by Koenigs *cinchene*.



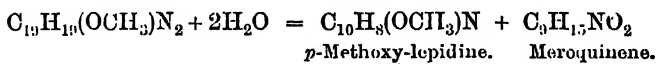
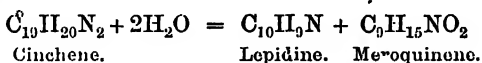
<sup>1</sup> Various papers in the *Berichte*, beginning with *Ber.*, 1880, 13, 286, to the present time.

Quinine behaves similarly and yields *quinene*;  $C_{19}H_{19}(OCH_3)N_2$ . To these two substances, cinchene and quinene, Koenigs and his collaborators<sup>1</sup> have devoted their attention with the object of establishing the structure of the 'second-half' of the molecule.

On prolonged boiling with strong hydrobromic acid, ammonia is split off and water taken up by both compounds, yielding *apocinchene* in one case and *apoquinene* in the other.

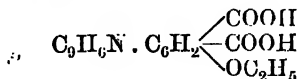


If, on the other hand, cinchene and quinene are heated with 25 per cent. phosphoric acid solution under pressure, two molecules of water are taken up, and lepidine and *p*-methoxy-lepidine are respectively formed, together with a second product which is common to both and is named *meroquinene*. The structure of meroquinene has a special significance, as it may be taken to represent the second-half of the two alkaloids.



**Structure of Apocinchene.** Koenigs succeeded, by oxidising the ethyl derivative of apocinchene, in resolving it step by step into three new products, which have been identified as derivatives of quinolyl-phenol.

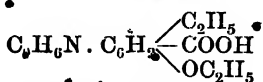
The first is known as *ethylapocinchenic acid*, the second as the lactone of *hydroxyethylapocinchenic acid*, and the third has been identified as *quinolylphenetole dicarboxylic acid*.



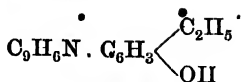
Again, if ethyl apocinchenic acid is boiled with hydrobromic acid, carbon dioxide and ethyl bromide are removed and *homapocinchene* is formed. The ethyl derivative of the latter yields on oxidation ethylhomapocinchenic acid; and if the silver salt is heated,  $\gamma$ -quinolyl-phenetole results. Finally,  $\gamma$ -quinolylphenetole is converted into  $\gamma$ -quinolylphenol with hydrobromic acid.

<sup>1</sup> *Annalen*, 1906, 347, 140.

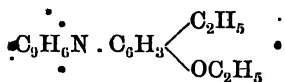
The series of changes are represented as follows :



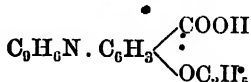
Ethylapocinchenic acid.



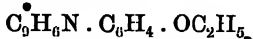
Homapocinchene.



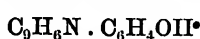
Ethylhomapocinchene.



Ethylhomapocinchenic acid.

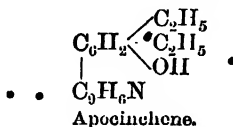


$\gamma$ -Quinolylphenetole.

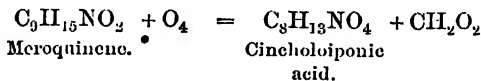


$\gamma$ -Quinolylphenol.

Putting the above facts together, the probable formula for apocinchene is that of a quinolyl diethylphenol.



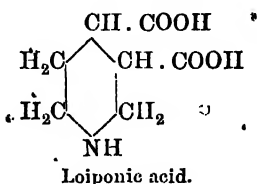
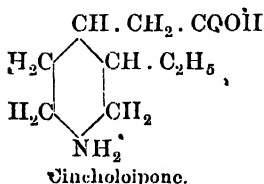
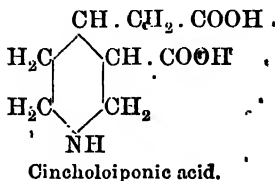
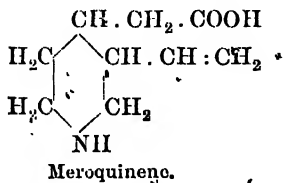
**Structure of Meroquinene.** In addition to the method already described for the preparation of meroquinene, Koenigs succeeded in obtaining it by the direct oxidation of cinchonine with chromic acid. By further oxidation with cold permanganate in presence of sulphuric acid meroquinene is converted into *cincholoiponic acid*,  $\text{C}_8\text{H}_{13}\text{NO}_4$ . Reduction, on the other hand, gives *cincholoipone* ( $\lambda\sigma\tau\omega\varsigma$  = residue).



From the latter Skraup obtained by careful oxidation with permanganate small quantities of a second acid which he termed *loiponic acid*,  $\text{C}_7\text{H}_{11}\text{NO}_4$ . All these compounds appear to contain a piperidine nucleus, the presence of which has been demonstrated in various ways. Thus, when meroquinene is heated with hydrochloric acid to  $240^\circ$  with or without the addition of mercuric chloride  $\gamma$ -methyl- $\beta$ -ethylpyridine is formed, whilst strong sulphuric acid converts cincholoiponic acid into  $\gamma$ -methylpyridine; finally, Koenigs showed that loiponic acid is transformed by heating with potash into an isomeric acid which is identical with synthetical hexahydrocinchomeyonic acid (piperidine- $\beta$ - $\gamma$ -dicarboxylic acid). The other reactions for meroquinene indicate that it is a secondary base, with an unsaturated side-chain (it forms an additive compound with bromine) and a carboxyl group (it forms an ester with alcohol).



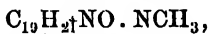
These facts taken together point to the following as the most probable formulae for meroquinene and its oxidation products,



It therefore follows that the 'second-half' is a piperidine nucleus, and, moreover, that it is attached to the  $\gamma$ -carbon of the quinoline nucleus by the carbon atoms of the  $\gamma$ -side-chain, since on oxidation meroquinene and cinchoninic acid are produced, each of which has a carboxyl in the  $\gamma$ -position. By the same process the hydroxyl group disappears, and must also form part of the  $\gamma$ -side-chain.

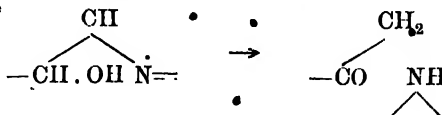
It will be seen from this summary that the structure of the 'second-half' and also the nature of the union between the piperidine and the quinoline nucleus is still uncertain, in addition to which the position of the hydroxyl group in the  $\gamma$ -side-chain is undetermined and the exact function of the  $\gamma$ -side-chain unknown. A certain amount of light has been thrown on these obscure points by the study of a substance known as *cinchotoxine*.

**Structure of Cinchotoxine.** The monoalkyl iodides of cinchonine produced by the direct action of the alkyl iodide, and in which the alkyl iodide has been shown to attach itself to the tertiary nitrogen of the 'second-half,' lose hydrogen iodide when decomposed by alkalis and form alkyl cinchonines. The methyl derivative,



obtained in this way was found by Miller and Rohde<sup>1</sup> to combine with phenylhydrazine and yield a hydrazone, whilst cinchonine itself does not give this reaction. If, however, cinchonine is submitted to the prolonged action of phenylhydrazine dissolved in dilute

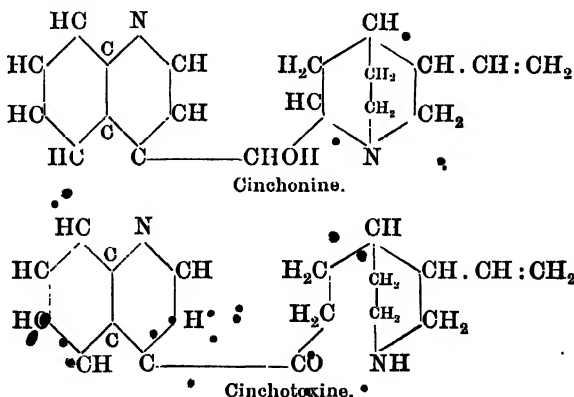
acetic acid at a temperature of  $100^{\circ}$ , combination ensues. The molecular rearrangement which evidently takes place was subsequently found to occur without the addition of phenylhydrazine by merely heating with acetic acid. The new compound is a base, isomeric with cinchonine, but possessing highly poisonous properties, on account of which it was named *cinchotoxine*. Quinine behaves in precisely the same manner and gives rise to *quinotoxine*. Cinchotoxine is a ketone and not an aldehyde, since it forms a hydrazone and oxime but does not reduce silver oxide. It is also a secondary base. How is the change from alcohol to ketone and from tertiary to secondary base to be explained? The change is probably tautomeric.



It would therefore appear that the link which binds the carbon to the nitrogen of the piperidine nucleus is dissolved. But this is not all. The hydroxyl group has also been shown to be attached to the  $\gamma$ -carbon of the quinoline nucleus, since it disappears in meroquinene and must therefore form part of the  $\gamma$ -side-chain.

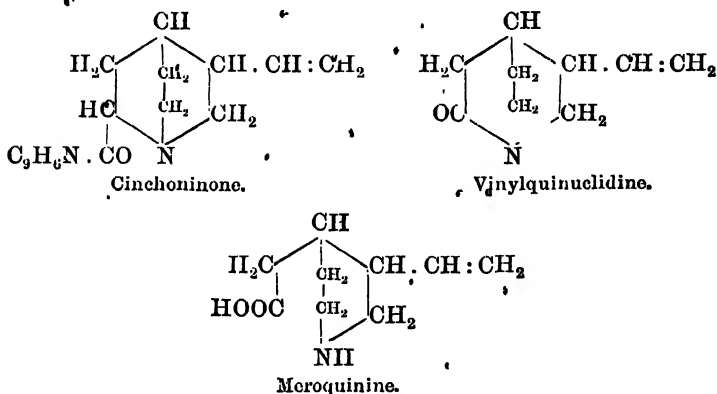
The formation of a ketone from cinchonine also means that the hydroxyl-carbon is already linked to a second carbon atom. This second carbon probably serves as the bond which unites the two halves of the molecule.

These facts led Koenigs to represent cinchonine and cinchotoxine by the following formulae:



This view of the structure has been confirmed by the work of

Rabe and his co-workers.<sup>1</sup> They find that on oxidation these alkaloids may be converted into ketones and at the same time two atoms of hydrogen are removed. Thus cinchonine,  $C_{15}H_{22}N_2O$ , and also cinchotoxine,<sup>2</sup> yield cinchoninone,  $C_{15}H_{20}N_2O$ . Cinchoninone reacts with amyl nitrite, forming an oximino-compound,  $C_{15}H_{13}N_2(OH)$ , to which the name *oximino-vinylquinuclidine* has been given, and at the same time cinchoninic acid is formed. On hydrolysis the former yields meroquinine. The relation between cinchoninone, vinylquinuclidine, and meroquinine, is easily explained by the aid of the following formulae:



As the other alkaloids of the group yield the same vinylquinuclidine oxime it follows that the difference lies in the quinoline half in quinine and quinidine, and that the relation subsisting between the two members of each pair of bases must be of a stereochemical character.<sup>3</sup>

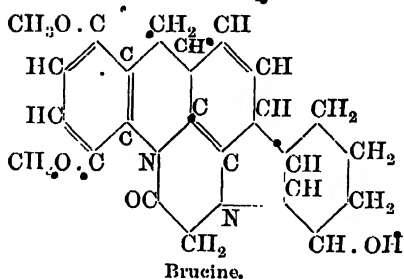
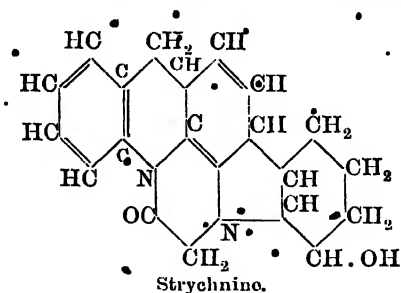
**The Strychnos Alkaloids** include strychnine and brucine, which are usually found together in several species of *strychnos*, the most important source being nux-vomica seeds and Ignatius beans. Both contain 2.5 to 3 per cent. of alkaloids, the proportion of the strychnine varying from one-half to two-thirds. Strychnine,  $C_{21}H_{22}N_2O_2$ , was discovered, as already stated, by Pelletier and Caventou in 1818, and its formula was correctly determined by Regnault in 1838. Its structure is not yet known with certainty, but sufficient information has been collected, chiefly by Tafel and

<sup>1</sup> *Annalen*, 1906, 350, 180; 1909, 365, 366; 1910, 373, 92; 1911, 382, 365.

<sup>2</sup> Rabe, *Ber.*, 1911, 44, 2088.

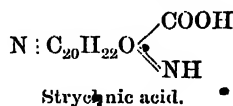
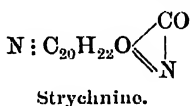
<sup>3</sup> Kaufmann and Huber, *Ber.*, 1913, 46, 2913.

Leuchs, to lead Perkin and Robinson<sup>1</sup> to propose the following formulae:

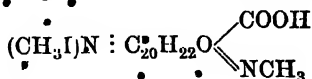


We reproduce the main arguments advanced by these two observers in support of the above formulae.

Strychnine contains two atoms of nitrogen, but is nevertheless a mono-acid tertiary base; for it combines with only one equivalent of acid and yields a mono-methiodide. The fact, established by Tafel,<sup>2</sup> that strychnine unites with a molecule of water when heated with alkalis forming an acid, *strychnic acid*, is taken to indicate the presence of a betaïne group, which becomes hydrolysed.



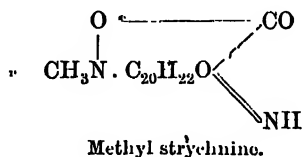
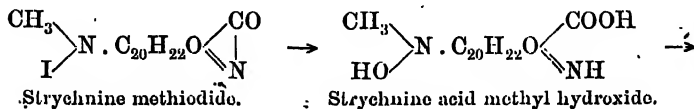
This will account for the non-basic character of the second nitrogen atom. Moreover, the presence of an NH group in strychnic acid is proved by the formation of a nitrosamine with nitrous acid and by that of a methiodide of methyl strychnine with methyl iodide.



<sup>1</sup> *Trans. Chem. Soc.*, 1910, 97, 309.

<sup>2</sup> *Annalen*, 1891, 264, 50.

Strychnine methiodide contains the betaine group, for it yields with baryta or silver oxide the methyl hydroxide of strychnic acid. The latter loses a molecule of water on heating and gives methyl strychnine. These changes are represented as follows:



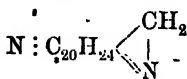
Methyl strychnine is a secondary base and on further methylation yields dimethylstrychnine, which forms a nitroso compound bearing a close resemblance to that of N-methyl tetrahydroquinoline (p. 286), seeing that it condenses with benzaldehyde in presence of zinc chloride, giving the leuco base of a green dye. Although no known quinoline derivative has so far been isolated, this and other facts give evidence of its presence. Moreover, strychnine contains a benzene nucleus, which probably forms part of the quinoline group, seeing that it can be readily nitrated and sulphonated.

Heated with hydriodic acid and phosphorus, strychnine loses one atom of oxygen and gains four atoms of hydrogen, yielding *desoxystrychnine*,  $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}$ , which still contains the betaine group, since it forms the corresponding strychnic acid with sodium ethoxide.<sup>1</sup>

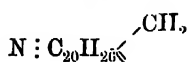
Many other reduction products are known, two of them dihydrostrychnoline,  $\text{C}_{21}\text{H}_{28}\text{N}_2$ , and strychnoline,  $\text{C}_{21}\text{H}_{26}\text{N}_2$ , being derived from desoxystrychnine by treatment with sodium and alcohol, whilst tetrahydrostrychnine,  $\text{C}_{21}\text{H}_{26}\text{O}_2\text{N}_2$ , are obtained from strychnine itself by the electrolytic reduction or by the palladium and hydrogen method (Part I, p. 162). These changes affect both oxygen atoms as well as the other parts of the molecule, and may be represented by the following formulae: <sup>2</sup>

<sup>1</sup> Tafel, *Annalen*, 1892, 268, 215.

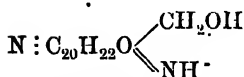
<sup>2</sup> Tafel, *Annalen*, 1898, 301, 303.



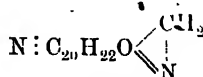
Strychnoline.



Dihydrostrychnoline.



Tetrahydrostrychnine.

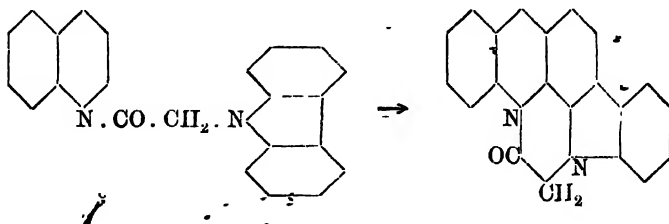


Strychnidine.

When strychnine is oxidised with chromic acid it gives an acid,  $\text{C}_{25}\text{H}_{17}\text{O}_2\text{N}_2 \cdot \text{COOH}$ , which on distillation with zinc dust yields carbazolē. Now as brucine, which is a dimethoxystrychnine, gives the same acid by the loss of the two methoxyl groups, it follows that the benzene nucleus is ruptured thus :



Perkin and Robinson point out that the carbazole group is probably an intrinsic part of the structure which, together with the quinoline group, forms the molecular framework. Now these two constituent groups contain  $\text{C}_{22}$ , and as the formula for strychnine is  $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$ , it follows that they are fused together, the nitrogen of the carbazole group forming the basic part. Such a fusion may be effected by linking the two nitrogen atoms by  $\text{CO} \cdot \text{CH}_2$ . But this would furnish two carbon atoms too many. The number can be reduced by supposing the two ring structures to be fused in the form of an acridine nucleus thus :



The various reduction products are represented by the complete or partial removal of oxygen and the reduction of unsaturated nuclei. Desoxystrychnine, which contains four hydrogen atoms more and

one oxygen atom less than strychnine, is represented by the addition of four hydrogen atoms to the second carbazole nucleus, whilst dihydrostrychnoline, the most highly-reduced product, contains in addition a  $\text{CH}_2$  in place of the  $\text{CO}$  group.

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